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Critical Influence of Timing of Administration of Granulocyte Colony-Stimulating Factor on Antibacterial Effect in Experimental Endocarditis Due to *Pseudomonas aeruginosa*

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The effect of human recombinant granulocyte colony-stimulating factor (hrG-CSF) in rabbits with aortic endocarditis due to *Pseudomonas aeruginosa* was investigated. hrG-CSF significantly increased the number of polymorphonuclear neutrophils in blood and in cardiac vegetations and the expression of the adhesin molecule CD11b on the surface of polymorphonuclear neutrophils compared with those of animals that had not received hrG-CSF. When treatment was started 72 h after bacterial challenge, hrG-CSF alone had no antibacterial effect and did not enhance the efficacy of ciprofloxacin when used in combination, even with the higher dosing regimen used (50 μg/kg of body weight subcutaneously every 12 h for 4 days), in terms of number of positive blood cultures, bacterial counts in vegetations, and survival. In contrast, when treatment was started 30 min prior to bacterial challenge, hrG-CSF (50 μg/kg injected every 12 h) decreased bacterial titers in vegetations 72 h later (6.5 ± 0.9 versus 7.9 ± 0.9 $\log_{10}$ CFU/g of vegetation for hrG-CSF and controls, respectively; $P = 0.0175$). However, this antibacterial effect was no longer detected after a 7-day treatment with hrG-CSF, and prophylactic administration of hrG-CSF did not increase the antibacterial effect of ciprofloxacin. We concluded that the antibacterial effect of hrG-CSF in experimental endocarditis was related to the timing of its administration since hrG-CSF demonstrated a significant but transient antimicrobial effect only when treatment was initiated before bacterial challenge.

Human polymorphonuclear neutrophils (PMNs) are the main phagocytic cells in the circulating blood and play a protective role against invading bacteria. Decreasing PMN counts during myelosuppressive chemotherapy or abnormal functions of PMNs are responsible for severe systemic and recurrent infections (24). Colony-stimulating factors (CSFs) are glycoproteins inducing proliferation and differentiation from bone marrow progenitor cells. Granulocyte CSF (G-CSF) is one of the cytokines which promotes the formation of granulocyte colonies and increases both the number and the activity of PMNs in peripheral blood (12, 14, 16, 21). Recently, a human recombinant G-CSF (hrG-CSF) was purified, cloned, and synthesized (23, 26). In patients with myelosuppression induced by chemotherapy for cancer, adjuvant treatment with hrG-CSF allows reduction in the incidence of fever with neutropenia, duration of hospitalization, and intravenous antibiotic therapy (4, 7). In human idiopathic neutropenia, hrG-CSF improves PMN counts and functions and reduces the number of infectious events (9). Experimentally, hrG-CSF prevents the reduction of the number of PMNs and restores host resistance to bacterial infections in mice rendered neutropenic by cyclophosphamide (13). However, the curative efficacy of hrG-CSF in established infection has not been demonstrated in either experimental or clinical settings.

Human left-sided bacterial endocarditis is a severe illness difficult to eradicate. Cellular host defenses have been assumed to be minimally operative within left-sided cardiac vegetations, since infected cardiac vegetations consist initially of relatively acellular platelet-fibrin matrix surrounding the bacterial colonies (1, 5). However, studies of patients and experimental animals with right-sided bacterial endocarditis document the presence of a more intense inflammatory cellular response within cardiac vegetations, suggesting a possible role for intravegetation host cell defenses in modifying the severity and antimicrobial responsiveness of this disease (20, 28). Furthermore, it has been shown in rabbits that intravegetation granulocyte influx appears to play a pivotal role in the trend towards spontaneous sterilization of tricuspid vegetations infected with *Pseudomonas aeruginosa*, since HN_2-induced granulocytopenia prevented the spontaneous clearance of *P. aeruginosa* from tricuspid vegetations observed in controls (2).

Therefore, we investigated whether hrG-CSF might improve the course of experimental infection in rabbits with left-sided endocarditis due to *P. aeruginosa*, by increasing the number and the activity of PMNs in blood and promoting intravegetation PMN influx, since aortic or mitral pseudomonal endocarditis is extremely difficult to cure with antibiotics alone (19). The influence of the timing of administration of hrG-CSF was studied, and the potential effect of hrG-CSF was investigated also in combination with ciprofloxacin, an antipseudomonal antibiotic with significant intracellular uptake which experimentally enhanced hematopoiesis, increased the number of circulating PMNs, and improved survival in neutropenic mice (10, 11).

MATERIALS AND METHODS

Organism. The study strain (serotype O11) was isolated from the blood of a patient with septic shock. This strain was susceptible to ciprofloxacin. The MIC and MBC were 0.5 and 1 μg/ml, respectively, as determined by the macrodilution method with an inoculum of 10^5 CFU/ml (18).

Media, antibiotics, and hrG-CSF. Mueller-Hinton broth and Mueller-Hinton agar (Sanofi Diagnostics Pasteur, Marnes-la-Coquette, France) were used. Antibiotics and hrG-CSF were provided by their manufacturers: ciprofloxacin

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(Bayer, Courbevoie, France), amikacin (Bristol Myers Squibb, Paris la Défense, France), and hrG-CSF (r-metHuG-CSF; 30 MU/ml) (Amgen, Thousand Oaks, Calif.).

Determination of peripheral blood PMN counts. One milliliter of blood was drawn with a polyethylene catheter from the marginal ear vein into a sodium heparin Microtainer (Becton Dickinson, Rutherford, N.J.). PMN counts were done with an STKS Coulter Counter (Coultronics, Hialeah, Fla.) which counts and sizes cells by analyzing volume, high-frequency conductivity, and laser light scatter as each cell passes through the flow cell. PMN counts were determined in preliminary studies 0, 3, 6, 24, 48, and 72 h after a single dose of 20 or 40 μg of hrG-CSF per kg of body weight in two groups of three uninfected rabbits to determine the optimal dose of hrG-CSF. In infected rabbits with endocarditis, blood was drawn during therapy 1.5 and 24 h after hrG-CSF injection from the first day of therapy to the day of sacrifice.

Experimental endocarditis. Investigations were performed with female New Zealand White rabbits (weight range, 2 to 2.5 kg). The animals were anesthetized by intramuscular injection with ketamine hydrochloride (15 mg/kg). Aortic endocarditis was induced by insertion of a polyethylene catheter through the right carotid artery into the left ventricle to induce the formation of vegetations (17). Twenty-four hours after catheter insertion, each rabbit was inoculated by marginal ear vein with 10^8 CFU of *P. aeruginosa* (range, 9×10^7 to 3×10^8 CFU) in 1 ml of 0.9% NaCl (day 1). This inoculum was prepared from an overnight culture, centrifuged at $500 \times g$ for 15 min, and resuspended in saline. Bacterial inoculation produced endocarditis in all rabbits with proper placement of the catheter. The catheter was left in place throughout the experiment.

Treatment regimens. (i) Curative regimens. Seventy-two hours after bacterial challenge (day 4), animals received one of the following regimens for 4 days (from days 4 to 7): ciprofloxacin, 40 mg/kg given intramuscularly every 12 h; hrG-CSF, 40 μg/kg subcutaneously every 24 h or 50 μg/kg subcutaneously twice daily (b.i.d.); or ciprofloxacin with 40 μg of hrG-CSF per kg subcutaneously every 24 h or 50 μg of hrG-CSF per kg subcutaneously b.i.d. Control animals remained untreated. All the animals were sacrificed at day 8, 12 h after the last injection for the treated animals.

(ii) Prophylactic regimens. In prophylactic regimen experiments, hrG-CSF administration was started 30 min before bacterial challenge (day 1). Animals received one of the following regimens: hrG-CSF at 50 μg/kg subcutaneously b.i.d. from days 1 to day 3 (i.e., six injections) or from days 1 to 7 (i.e., 14 injections) or 50 μg of hrG-CSF per kg subcutaneously b.i.d. from days 1 to 7 plus ciprofloxacin given as described above (40 mg/kg intramuscularly every 12 h from days 4 to 7). Control animals remained untreated. Animals were sacrificed at day 4 or at day 8, 12 h after the last injection for the treated animals.

Sacrifice. At the time of sacrifice, approximately 20% of the rabbits were used for histologic studies and 80% were used for intravegetation bacterial density determination. In the latter case, the hearts were removed and the vegetations of each animal were excised, pooled, and weighed. They were homogenized in 0.5 ml of sterile saline, and 0.1-ml portions were quantitatively subcultured on agar plates for 24 h. The lower limit of detection by this method was 10^2 CFU/g. At the time of sacrifice, 1 ml of blood was cultured in 9 ml of Mueller-Hinton broth, and 0.2 ml of blood from each rabbit used for CFU determination was spread on the culture.

Determination of CD11b expression at the PMN surface. Three milliliters of blood was sampled in lithium heparin the day prior to sacrifice. Whole-blood samples were either kept on ice or incubated with *N*-formyl-methionyl-leucyl-phenylalanine (fMLP) (10^{-6} mol/liter) (Sigma Chemical Co., St. Louis, Mo.) or phosphate-buffered saline (PBS) (Pharmacia Fine Chemicals, Uppsala, Sweden) at 37°C for 5 min. Then 40 μl of whole blood was incubated with 10 μl of a monoclonal antibody directed against the CD11b subunit of rabbit CD11b-CD18 integrin (monoclonal unlabeled CD11b antibody; Serotec, Oxford, England) for 30 min at 4°C. After being washed with cold PBS ($400 \times g$ for 5 min), the cells were incubated with fluorescein isothiocyanate-conjugated goat anti-mouse antibody (Nordic Laboratories, Tilburg, The Netherlands) for 30 min on ice. After one washing with ice-cold PBS, erythrocytes were lysed with Facs lysing solution (Becton Dickinson) and the leukocytes were resuspended in 1% paraformaldehyde-PBS and kept on ice until flow cytometry analysis.

Flow cytometry analysis. A Becton Dickinson FACScan (Immunocytometry Systems, San Jose, Calif.) with a 15-mW, 488-nm argon laser was used. Forward and side scatter was used to identify the granulocyte population and to gate out other cells and debris. The green fluorescences of fluorescein isothiocyanate-conjugated goat anti-mouse antibody were recorded from 515 to 545 nm. In all cases, unstained cells were run and the photomultiplier settings were adjusted so that the unstained cell population appeared in the lower left-hand corner of the fluorescence display. Ten thousand events were counted per sample, and the fluorescence pulses were amplified by 4-decade logarithmic amplifiers. All the results were obtained with the use of a constant photomultiplier gain value. The data were analyzed with LYSIS II software (Becton Dickinson), and the median fluorescence intensity was used to quantitate the responses. The effect of fMLP on CD11b expression on the PMN surface was calculated by using the median fluorescence intensity of stimulated cells.

Histological studies. Immediately after removal, entire hearts were fixed in 10% formaldehyde for 24 h, rinsed in PBS, and finally embedded in paraffin. Formaldehyde-paraffin sections (4 μm thick) were stained with hematein-eosin-safran and Gram stain. Global cell infiltration, PMN infiltration, and necrosis were examined by two anatomopathologists blinded as to the regimens corresponding to the vegetations studied and scored in a semiquantitative manner from 0 to +++ according to the intensity of each parameter, as previously described (25).

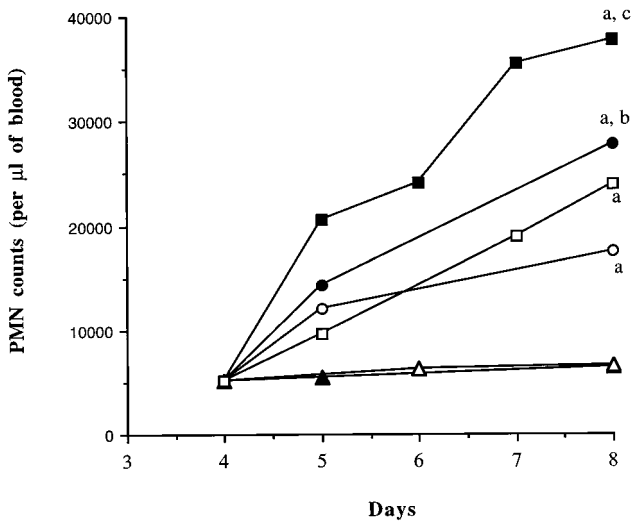

FIG. 1. Peripheral blood PMN counts for rabbits with endocarditis due to *P. aeruginosa* treated for 4 days (days 4 to 7) with ciprofloxacin at 40 mg/kg intramuscularly every 12 h (△), hrG-CSF at 40 μg/kg every 24 h (○), ciprofloxacin and hrG-CSF at 40 μg/kg every 24 h (●), hrG-CSF at 50 μg/kg every 12 h (□), or ciprofloxacin and hrG-CSF at 50 μg/kg every 12 h (■) or untreated (controls) (▲). The animals were sacrificed at day 8. Each point is the mean for two to seven rabbits. a, $P < 0.01$ versus controls and ciprofloxacin-treated animals; b, $P < 0.01$ versus results for hrG-CSF at 40 μg/kg every 24 h; c, $P < 0.001$ versus results for hrG-CSF at 50 μg/kg every 12 h.

Statistical analysis. All data were expressed as means ± standard deviations. The means of more than two groups (bacterial counts in vegetations, counts of PMNs in blood, and CD11b expression on the PMN surface) were compared by analysis of variance followed by the Scheffe test for multiple comparisons. When only two groups were compared, an unpaired *t* test was performed.

RESULTS

PMN counts in rabbits. After a single dose of 20 or 40 μg of hrG-CSF per kg in noninfected rabbits, PMN counts increased and peaked at 24 h. For the 40-μg/kg dose, PMN counts at 24 h were significantly higher than before the injection of hrG-CSF (12,030 ± 2,310 versus 2,060 ± 1,850 PMNs per μl, 24 h after and before the injection of 40 μg of hrG-CSF per kg, respectively; $P = 0.004$). Seventy-two hours after injection, PMN counts returned to their baseline levels. Since the 40-μg/kg dose increased the PMN counts after a single injection, a regimen of 40 μg of hrG-CSF per kg once daily was chosen for initial experimental studies.

Peripheral blood PMN counts for rabbits with experimental endocarditis progressively increased during therapy with hrG-CSF compared with those of controls and ciprofloxacin-treated animals (Fig. 1). At day 8, PMN counts for rabbits that received 40 μg of hrG-CSF per kg once daily were significantly higher than those for infected controls ($P < 0.01$) and ciprofloxacin-treated animals ($P < 0.01$) (16,330 ± 3,410 [$n = 6$] versus 6,170 ± 2,590 [$n = 6$] and 7,260 ± 1,690 [$n = 6$] PMNs per μl for hrG-CSF-treated animals versus infected controls and ciprofloxacin-treated animals, respectively). This result was even more pronounced with the higher dose of hrG-CSF (Fig. 1). Furthermore, PMN counts at day 8 for rabbits that received hrG-CSF in combination with ciprofloxacin were significantly increased compared with PMN counts for rabbits that received hrG-CSF alone (27,900 ± 16,000 and 44,875 ±

TABLE 1. CD11b expression and granularity of PMNs from whole blood of rabbits infected with *P. aeruginosa* and uninfected rabbits

Group (no. of samples)	Basic CD11b expression at the surface of PMNs from whole blood[a]	Basic right-angle scatter of PMNs from whole blood[b]
Uninfected (8)	18 ± 4	452 ± 67
Infected (8)	25 ± 15	478 ± 82
hrG-CSF, 40 μg/kg (12)	47 ± 12[c]	276 ± 75[d]

[a] Median fluorescence intensity (means ± standard deviations are shown).
[b] PMN right-angle scatter, analyzed by flow cytometry, is related to PMN granularity. Values shown are means ± standard deviations.
[c] $P < 0.01$ versus uninfected and infected rabbits.
[d] $P < 0.001$ versus uninfected and infected rabbits.

23,300 PMNs per μl, with 40 μg of hrG-CSF per kg once daily and 50 μg of hrG-CSF per kg b.i.d. in combination with ciprofloxacin, respectively; $P < 0.01$ and $P < 0.001$ in comparison with hrG-CSF alone, respectively).

Expression of the CD11b adhesin molecule at the PMN surface. As shown in Table 1, the level of basic CD11b expression of PMNs from circulating blood of infected rabbits treated with hrG-CSF was significantly higher than that of uninfected controls or infected animals that did not receive hrG-CSF ($P < 0.01$). Ex vivo stimulation with fMLP significantly increased the expression of the CD11b molecule at the surface of PMNs from healthy rabbits, infected rabbits, and hrG-CSF-treated rabbits (median fluorescence intensity ratios after and before stimulation with fMLP, 1.82 ± 0.58, 1.88 ± 0.40, and 1.52 ± 0.46 for healthy, infected, and hrG-CSF-treated rabbits, respectively; $P < 0.01$ for the three groups). This increase was not significantly different among the three groups ($P > 0.20$), indicating normal expression of the CD11b-CD18 molecules after PMN stimulation for the three groups of rabbits. In vitro, CD11b expression of PMNs in whole blood from healthy rabbits increased by 40% after incubation at 37°C with hrG-CSF (50 μg/ml) for 10 min and then fMLP for 10 min compared with that of PMNs incubated with hrG-CSF or fMLP alone (data not shown).

CD11b-CD18 molecules are located in PMN granules, and their increased expression at the cell surface after PMN stimulation involves intracellular translocation associated with degranulation. Therefore, using flow cytometry, we analyzed the PMN right-angle scatter, which is related to PMN granularity. The right-angle scatter was significantly decreased in infected animals treated with hrG-CSF compared with that for uninfected or infected controls ($P < 0.001$), as shown in Table 1.

Experimental endocarditis. (i) Curative regimens. Results of the different 4-day treatment regimens for rabbits infected by *P. aeruginosa* are shown in Table 2. hrG-CSF alone did not modify bacterial counts in vegetations and did not increase the efficacy of ciprofloxacin when used in combination, whatever the dosing regimen (40 μg/kg once daily or 50 μg/kg b.i.d.). Blood cultures remained positive at the time of sacrifice for all rabbits treated with hrG-CSF alone, whatever the dose, and to the same extent as for controls but were constantly negative with all regimens including ciprofloxacin. In addition, the survival of the animals was not increased by administration of hrG-CSF (Table 2).

TABLE 3. Results of different regimens using hrG-CSF started 30 min before bacterial challenge in rabbits with aortic endocarditis due to *P. aeruginosa*

Treatment	Mean $\log_{10}$ CFU/g of vegetations ± SD at:	
	Day 4	Day 8[a]
None (controls)	7.9 ± 0.9 (6)	8.4 ± 1.0 (9)
hrG-CSF, 50 μg/kg b.i.d. from days 1 to 7	6.5 ± 0.9[b] (7)	9.0 ± 1.6 (6)
Ciprofloxacin from days 4 to 7		6.2 ± 0.5[c] (4)
hrG-CSF, 50 μg/kg b.i.d. from days 1 to 7 + Ciprofloxacin from days 4 to 7		6.1 ± 0.4[c] (6)

[a] Animals were sacrificed 12 h after the last injection.
[b] $P = 0.0175$ versus controls.
[c] $P < 0.001$ versus controls and hrG-CSF alone.

(ii) Prophylactic regimens. In contrast to what was observed with curative regimens, administration of hrG-CSF alone at 50 μg/kg b.i.d. beginning 30 min before bacterial challenge significantly reduced bacterial titers in vegetations after 3 days of treatment compared with those of controls (6.5 ± 0.9 versus 7.9 ± 0.9 $\log_{10}$ CFU/g of vegetation for hrG-CSF and controls, respectively; $P = 0.0175$). However, no antibacterial effect was observed 4 days later, after a 7-day regimen of hrG-CSF at 50 μg/kg b.i.d. (Table 3). Finally, prophylactic administration of hrG-CSF did not enhance the bactericidal effect of ciprofloxacin (Table 3).

Histological studies by light microscopy. Animals that received 40 μg of hrG-CSF per kg once a day had increased cellular (+++) and PMN (++ or +++) infiltrations in vegetations compared with controls (+ or ++), as shown in Fig. 2.

TABLE 2. Results of different 4-day treatment regimens started 72 h after bacterial challenge in rabbits with aortic endocarditis due to *P. aeruginosa*

Treatment	No. of positive blood cultures/total	Log_{10} CFU/g of vegetations (mean ± SD)	No. of animals with sterile vegetations/total	No. of survivors/total no. of animals treated[a]
None (controls)	8/8	8.3 ± 1.0	0/8	10/12
hrG-CSF				
40 μg/kg once a day	7/7	8.7 ± 0.4	0/7	8/9
50 μg/kg b.i.d.	3/3	8.8 ± 0.4	0/3	1/3
Ciprofloxacin	0/10	4.8 ± 1.5[b]	0/10	15/15
Ciprofloxacin with hrG-CSF at:				
40 μg/kg once a day	0/10	4.7 ± 1.1[b]	0/10	11/12
50 μg/kg b.i.d.	0/4	4.8 ± 1.9[b]	0/4	4/4

[a] Some of the treated animals were used for histological studies only. Thus, the total number of treated animals was higher than the number of animals for which bacteriologic data are available.
[b] $P < 0.0001$ versus controls and hrG-CSF-treated rabbits.

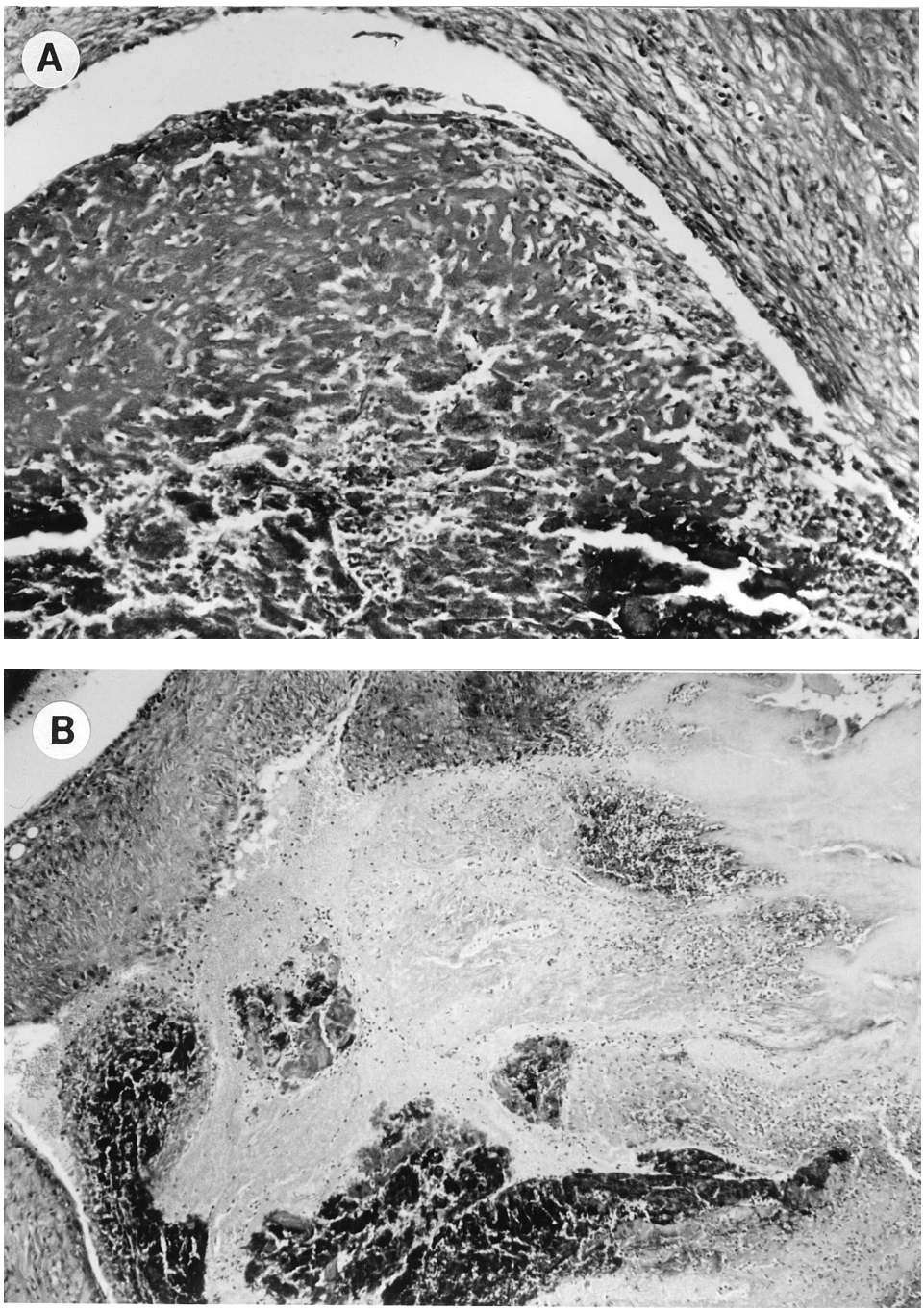

FIG. 2. (A) Vegetation from an infected and untreated rabbit, showing large areas of necrosis and fibrin with foci of PMNs scattered. (B) Vegetation from an infected rabbit treated with hrG-CSF alone. Necrosis is less abundant than in controls, but the PMN population is greatly enhanced. The PMNs are often altered, with many important caryorhexis. Magnification, ×400.

DISCUSSION

The major finding of the present study was the critical importance of the timing of administration of hrG-CSF for its antibacterial activity.

When administration was started 72 h after bacterial challenge, hrG-CSF alone had no bactericidal effect and did not enhance the activity of ciprofloxacin in terms of positive blood cultures, bacterial counts in vegetations, number of sterile vegetations, and animal survival. This result confirms the findings of the only previous study that investigated the effect of hrG-CSF for therapy of established infection in combination with an antibiotic (6). In that study, hrG-CSF was combined with ceftriaxone for experimental endocarditis due to *Staphylococcus aureus* in rabbits. hrG-CSF did not increase the bactericidal activity of ceftriaxone. However, the activation of PMNs was not determined, and a low dose of hrG-CSF was used (6). In our study, the lack of efficacy of hrG-CSF was not due to a species specificity, for several reasons. (i) In vitro, hrG-CSF

effectively primed PMNs from healthy rabbits, a result further triggered by fMLP. (ii) PMNs from rabbits treated with hrG-CSF had enhanced expression of the adhesin molecule CD11b and decreased granularity compared with PMNs from uninfected or infected rabbits that had not received hrG-CSF. (iii) PMN counts were significantly increased with a dose-dependent response in peripheral blood from uninfected and infected rabbits treated with various doses of hrG-CSF. Therefore, hrG-CSF was biologically active against rabbit PMNs. In addition, the absence of in vivo efficacy of hrG-CSF in the present study was not related to an insufficient dosage, since enhanced CD11b expression and increased numbers of PMNs in blood were already achieved with the 40-μg/kg daily dose. With a higher dosage (50 μg/kg b.i.d.), higher peripheral PMN counts were obtained, but this regimen was also ineffective in terms of bactericidal effect. Finally, a substantial infiltration of PMNs in rabbits receiving hrG-CSF, which could be related to the increased expression of adhesin molecules at the PMN surface, was observed. However, in situ activation of PMNs could not be determined for aortic vegetations from rabbits treated with different regimens.

When hrG-CSF treatment was initiated 30 min before bacterial challenge, a significant reduction in bacterial titers in cardiac vegetations compared with those of controls was achieved 72 h later. However, this effect was transient, since the counts of bacteria in vegetations were comparable to those of controls 4 days later, i.e., after a 7-day treatment. Several studies previously proved that hrG-CSF could protect healthy or immunocompromised animals (e.g., newborns, animals with burn wounds, and neutropenic or alcoholic animals) when used before or at the time of infection (3, 8, 13, 15, 22, 27).

Several factors might explain the influence of the timing of administration of hrG-CSF on its antibacterial activity. The first one is the delay in the increase in PMN counts with the curative regimen as opposed to the prophylactic regimen. This point might be important, because previous studies suggested that changes in granulocyte counts lag behind changes in peripheral blood PMN counts by several days in experimental endocarditis (2). An additional explanation for the different activities of hrG-CSF according to the timing of its administration may be the importance of the in vivo bacterial inoculum present at the site of infection when curative therapy with hrG-CSF was started ($\sim 10^8$ CFU/g of vegetation), in contrast with prophylactic regimens. A third explanation may be related to the distribution of granulocytes in vegetations. In prophylactic regimens, hrG-CSF is given when vegetations are small and relatively acellular (5); therefore, prophylactic administration of hrG-CSF may induce a protective effect for a few days by increasing PMN counts in the vegetations before or at the time of bacterial colonization. This may explain the delay in bacterial growth observed in our study 72 h after bacterial challenge in hrG-CSF-pretreated animals compared with controls. In contrast, in large vegetations, the uneven distribution of inflammatory cells in aortic vegetations compared with tricuspid vegetations explains why granulocytes are rarely seen in proximity to bacterial colonies (2) and may explain why, despite prolonged therapy with hrG-CSF, no antibacterial effect was observed at day 8, when vegetations became larger as the infection progressed.

Although ciprofloxacin is not the standard therapy for pseudomonal endocarditis, this antibiotic was chosen for our investigations because previous experimental studies demonstrated that ciprofloxacin enhanced hematopoiesis, increased the number of circulating PMNs, and improved survival in neutropenic mice (10, 11). Therefore, a synergistic antibacterial effect might be expected for a combination of ciprofloxacin and hrG-CSF, with a combined effect on PMNs. We indeed found a synergistic hematopoietic effect with these two molecules, since animals that received ciprofloxacin in combination with hrG-CSF had higher counts of PMNs in peripheral blood than rabbits that received hrG-CSF alone. However, this was not paralleled with an increased antibacterial effect of the combination compared with that of ciprofloxacin alone.

In conclusion, this study demonstrates that the antibacterial effect of hrG-CSF is related to the timing of its administration, since only prophylactic administration of hrG-CSF showed a significant but transient antimicrobial effect. Further in vivo studies with other experimental models of infection are needed to determine whether the lack of curative antimicrobial efficacy of hrG-CSF reported here is specific to endocarditis.

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