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Granulocyte-Macrophage Colony-Stimulating Factor in *Staphylococcus aureus*-Induced Arthritis

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Granulocyte-macrophage colony-stimulating factor (GM-CSF) is a cytokine that is able to increase not only the production of phagocytic cells but also their efficacy with respect to, e.g., bactericidal properties. In this study, we wanted to analyze the impact of GM-CSF on experimental *Staphylococcus aureus*-induced arthritis. For that purpose, mice were administered GM-CSF before and after bacterial inoculation. Although there was an increase in the total number of leukocytes as well as in the granulocyte fraction, there was no favorable effect on the severity of arthritis or on survival rates. There were no obvious differences between the GM-CSF-pretreated animals and controls with regard to growth of staphylococci in joints and kidneys 4 days after the bacterial inoculation. In contrast, mice that had been pretreated with GM-CSF prior to bacterial inoculation showed approximately four times lower numbers of bacteria in their blood 24 h later. These results, along with those of our previous studies, suggest that on the one hand the granulocyte is the main protective cell during the course of *S. aureus* infection but that on the other hand, upregulation of granulocyte-macrophage production will not exert any additional protective effects with respect to tissue injury.

We have previously described a murine model of hematogenously induced *Staphylococcus aureus* sepsis and arthritis. Mice inoculated with *S. aureus* LS-1 develop clinical arthritis with irreversible joint damage within a few days of infection (5). In this model, large numbers of granulocytes can be observed both in the circulation and locally, in the inflamed synovium, within 24 h after bacterial inoculation. When the animals were rendered neutropenic prior to bacterial infection, they displayed severe septicemia and died because of septic shock (28).

Granulocyte-macrophage colony-stimulating factor (GM-CSF) stimulates the survival, proliferation, and differentiation of myeloid cells and their precursors, particularly neutrophilic and eosinophilic granulocytes and monocytes/macrophages (23, 26). This cytokine mimics some of the actions of gamma interferon since it activates macrophages to produce tumor necrosis factor alpha (TNF-α) and interleukin-1 (IL-1) (1, 14). Recombinant murine GM-CSF (rmGM-CSF) has been applied in different experimental infections (7, 29), in certain instances displaying protective properties (2, 13, 15) and in others lacking any effect (3, 27).

In the present study, we investigated whether increased levels of neutrophils would be beneficial for the outcome of *S. aureus*-induced arthritis. For that purpose, we treated mice with rmGM-CSF before and after intravenous inoculation of bacteria.

S. aureus LS-1 was originally isolated from a swollen joint of a spontaneously arthritic NZB/W mouse (6). Mice were injected intravenously (i.v.) with an amount of *S. aureus* corresponding to 2.5×10^7 CFU per mouse. Ten male 6- to 7-week-old NMRI mice (B&K Universal AB, Stockholm, Sweden) were injected intraperitonally (i.p.) with 500 ng of rmGM-CSF (Genzyme, Cambridge, Mass.) 24 and 4 h before bacterial inoculation and then once a day for another 3 days. Another 10 mice received 500 ng of rmGM-CSF 72 h after injection of bacteria and were then supplied with GM-CSF once daily for another 4 days. As a control, 10 mice received phosphate-buffered saline (PBS). The doses of GM-CSF were chosen according to earlier studies (2, 19, 23). Arthritis was defined as visible joint erythema and/or swelling of at least one joint. To evaluate the intensity of arthritis, a clinical scoring system of 0 to 3 points for each limb was used. An arthritic index was constructed by adding the scores from all four limbs of each mouse (4).

In a second experiment, eight mice, 7 to 8 weeks old, received 500 ng of rmGM-CSF 48, 24, and 2 h prior to i.v. inoculation of 3×10^7 CFU of *S. aureus* and then once daily for another 2 days. Eight mice were treated with PBS and used as controls. Total leukocyte counts were determined in a hemacytometer (Toa Medical Electronics, Kobe, Japan). Blood smears were prepared and then stained by the May-Grunewald-Giemsa method for differential counts. Total leukocyte and neutrophil counts in mice pretreated with rmGM-CSF were determined 24 h and 4 days after bacterial inoculation. In noninfected animals, blood sampling was performed 8 and 20 h after administration of GM-CSF.

Twenty-four hours after i.v. inoculation of *S. aureus*, growth of staphylococci in the blood was determined. Four days after inoculation, and thus 48 h after the last GM-CSF administration, the mice were sacrificed. Bacterial samples from the joints and homogenized kidneys were plated on blood agar plates. In addition, peripheral blood cells were stained with a monoclonal antibody recognizing mouse F4/80 antigen expressed on murine monocytes/macrophages (Serotec, Oxford, Great Britain). The cells were analyzed by using a FACScan flow cytometer (Becton Dickinson, Mountain View, Calif.).

Sera were collected 24 h, 48 h, and 4 days after bacterial inoculation to determine circulating levels of GM-CSF. Sera from noninfected and infected mice ($n = 2$ to 5) were obtained on days 5 and 11 after bacterial inoculation. Levels of serum GM-CSF were determined by using a GM-CSF enzyme-linked immunosorbent assay kit (Endogen, Cambridge, Mass.).

The differences between nonparametric values in the groups were tested for significance by use of the Mann-Whitney test. The differences between groups with respect to the occurrence of arthritis were analyzed by use of the chi-square test. Values are reported as means ± standard errors of the means.

Naive mice did not display detectable GM-CSF levels in their

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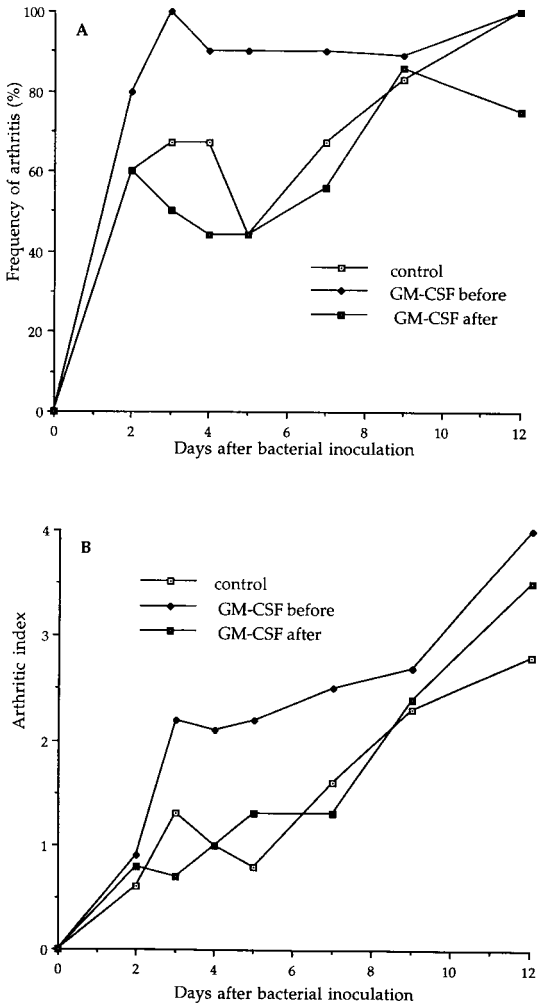

FIG. 1. Effects of administration of rmGM-CSF on the frequency (A) and severity (B) of *S. aureus*-induced arthritis. The differences are not statistically significant.

circulations. Five days after inoculation with *S. aureus*, the mice exhibited increased levels of GM-CSF in their sera (29 ± 26 pg/ml). Eleven days after the bacterial inoculation, there were no detectable levels of circulating GM-CSF (<5 pg/ml). Between days 3 and 9 after inoculation of bacteria, the GM-CSF-pretreated mice exhibited a slightly more prevalent and a more severe arthritis than the control mice injected with PBS as well as the mice treated with GM-CSF 2 days after bacterial inoculation (Fig. 1). Thus, 4 days after the inoculation, 90% of the GM-CSF-pretreated group displayed clinical signs of arthritis and an arthritic index of 2.1, compared to a 67% arthritis frequency and an arthritic index of 1.0 in the controls. Mice given GM-CSF 2 days after bacterial inoculation exhibited a frequency and a severity of arthritis similar to those of the controls. There were no significant differences between the three groups with regard to mortality or weight development (data not shown). The mice were sacrificed 12 days after infection, and at that time between 50 and 60% of the mice in each group were dead. This outcome is different from results obtained by Frenck et al. (13), who found that giving rmGM-CSF 6 h prior to inoculation of a lethal dose of *S. aureus* significantly improved the survival of neonatal rats. Differences in age and species of experimental animals could explain the observed discrepancies.

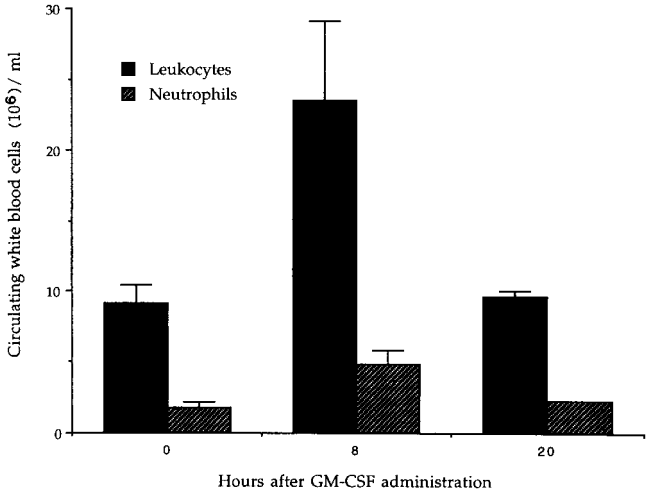

FIG. 2. Peripheral blood leukocyte and granulocyte counts in healthy NMRI mice (n = 2 to 7) at different periods after administration of rmGM-CSF. The differences are not statistically significant.

Our control experiments revealed that total numbers of circulating neutrophils were elevated almost threefold 8 h after GM-CSF injection into healthy mice. After 20 h, the mice had a slightly less than twofold increase in neutrophil numbers (Fig. 2). In the infected animals pretreated with GM-CSF, there was also an increase in the number of granulocytes compared to the PBS-pretreated controls both at 24 h ($5.8 \times 10^6 \pm 2.75 \times 10^6$/ml versus $4.1 \times 10^6 \pm 1.25 \times 10^6$/ml) and at 4 days ($11.3 \times 10^6 \pm 5.5 \times 10^6$/ml versus $7.1 \times 10^6 \pm 1.3 \times 10^6$/ml) after bacterial inoculation. In contrast, increases in numbers of monocytes were not observed.

Bacterial counts in whole blood 24 h after *S. aureus* inoculation were 130 ± 70 CFU/ml in the GM-CSF-pretreated group and 640 ± 300 CFU/ml in the control group (Fig. 3). With respect to bacterial growth in joints and kidneys, there were no obvious differences between the GM-CSF-pretreated group and the control animals.

Our present results indicate that despite an increase in the total number of leukocytes as well as in the granulocyte fraction as a consequence of GM-CSF treatment, there is no measurable favorable effect on the severity of arthritis or survival rates during *S. aureus* infection. A potential explanation for this finding is a deficient phagocytic and/or intracellular killing capacity of the

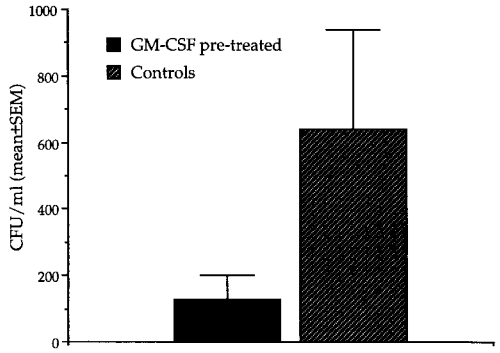

FIG. 3. Numbers of staphylococci, expressed as CFU per milliliter, in whole blood 24 h after inoculation with *S. aureus* of mice pretreated with GM-CSF (n = 4) and in controls (n = 4). The differences are not statistically significant. SEM, standard error of the mean.

newly synthesized polymorphonuclear cell/monocyte population. This does not seem to be the case, since GM-CSF (i) increases rather than decreases the phagocytic capacity of neutrophils (9); (ii) increases the potential of these cells to undergo respiratory burst (10), an important way to mediate intracellular killing (16); and, finally, (iii) increases the life span of neutrophils (14).

Alternatively, abnormally high levels of neutrophils/monocytes, triggered by the administration of GM-CSF, would have increased the host inflammatory response to staphylococcal infection. Highly activated neutrophils, e.g., those engaged in engulfing and extracellular killing of *S. aureus*, will release the toxic contents of their granules to the surrounding environment (24). These toxic moieties contain highly phlogistic substances, such as reactive oxygen and nitrogen species and proteolytic enzymes, that are capable of triggering tissue destruction and death (24, 25). Indeed, our results indicate that GM-CSF given before inoculation of *S. aureus* increased somewhat the frequency and severity of early arthritis compared to the controls and to mice provided with GM-CSF later in the course of the infection (Fig. 1). In this respect, our data agree with those of Frenck et al. (13) indicating that pretreatment of animals with GM-CSF renders an increase in neutrophil activation and enables a more powerful inflammatory response.

We have recently demonstrated an increase in production of TNF-α, IL-6, and gamma interferon during *S. aureus* infection (4, 30). These cytokines are able to greatly extend the survival of granulocytes (8). Interestingly, GM-CSF is able to augment the release of TNF (21), a major proinflammatory mediator involved in the destruction of joints in both aseptic (12) and septic (17) arthritis. Since neutrophils not only respond to GM-CSF but are also able to produce this cytokine (18), it is clearly possible that an increase in the number and the functional capacity of these cells would lead to the production of proinflammatory, tissue-injuring cytokines such as TNF and IL-1 (11, 20, 22). In this regard, during the natural course of *S. aureus* infection, increased levels of GM-CSF are found early during the disease.

In this study, we showed that treatment with GM-CSF of nonimmunocompromised subjects harboring *S. aureus* infection does not provide any significant protection despite the resulting increase in the production of granulocytes. We believe that this outcome may be due to the proinflammatory effects of this cytokine attributable to upregulation of phagocytic function.

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