

## A Randomized, Placebo-Controlled Trial of Recombinant Human Granulocyte Colony-Stimulating Factor Administration in Newborn Infants With Presumed Sepsis: Significant Induction of Peripheral and Bone Marrow Neutrophilia

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Host defenses in the human neonate are limited by immaturity in phagocytic immunity. Such limitations seem to predispose infected newborns to neutropenia from an exhaustion of the neutrophil reserve. Among the critical defects thus far identified in neonatal phagocytic immunity is a specific reduction in the capacity of mononuclear cells to express granulocyte colony-stimulating factor (G-CSF) after stimulation. However, the safety, pharmacokinetics, and biological efficacy of administration of recombinant human (rh)G-CSF to infected human newborns to compensate for this deficiency is unknown. Forty-two newborn infants (26 to 40 weeks of age) with presumed bacterial sepsis within the first 3 days of life were randomized to receive either placebo or varying doses of rhG-CSF (1.0, 5.0, or 10.0  $\mu\text{g}/\text{kg}$  every 24 hours [36 patients] or 5.0 or 10.0  $\mu\text{g}/\text{kg}$  every 12 hours [6 patients]) on days 1, 2, and 3. Complete blood counts with differential and platelet counts were obtained at hours 0, 2, 6, 24, 48, 72, and 96. Circulating G-CSF concentrations were determined at hours 0, 2, 6, 12, 14, 16, 18, 24, and 36. Tibial bone marrow aspirates were obtained after 72 hours for quantification of the bone marrow neutrophil

storage pool (NSP), neutrophil proliferative pool, granulocyte progenitors, and pluripotent progenitors. Functional activation of neutrophils (C3bi expression) was determined 24 hours after rhG-CSF or placebo administration. Intravenous rhG-CSF was not associated with any recognized acute toxicity. RhG-CSF induced a significant increase in the blood neutrophil concentration 24 hours after the 5 and 10  $\mu\text{g}/\text{kg}$  doses every 12 and 24 hours and it was sustained as long as 96 hours. A dose-dependent increase in the NSP was seen following rhG-CSF. Neutrophil C3bi expression was significantly increased at 24 hours after 10  $\mu\text{g}/\text{kg}$  every 24-hour dose of rhG-CSF. The half-life of rhG-CSF was  $4.4 \pm 0.4$  hours. The rhG-CSF was well tolerated at all gestational ages treated. The rhG-CSF induced a significant increase in the peripheral blood and bone marrow absolute neutrophil concentration and in C3bi expression. Future clinical trials aimed at improving the outcome of overwhelming bacterial sepsis and neutropenia in newborn infants might include the use of rhG-CSF.

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**B**ACTERIAL SEPSIS occurs in 1 to 10 per 1,000 term infants and is three to four times more common in infants delivered prematurely.<sup>1,2</sup> The mortality rate for neonates with bacterial sepsis varies between 15% and 75% depending on the virulence of the organism, associated complications, and gestational age.<sup>1,3</sup> Neutropenia commonly occurs in neonates with sepsis<sup>4-6</sup> and is associated with increased risk of death.<sup>7</sup> The kinetic and molecular explanations for the neutropenia observed in neonates with sepsis have been the subjects of previous investigations. We previously observed quantitative deficiencies in myelopoiesis in the neonatal rat.<sup>8-11</sup> These deficiencies include a relatively small supply of granulocyte progenitor cells (colony-forming unit granulocyte-macrophage) (CFU-GM), a small neutrophil storage pool (NSP) (polymorphonuclear leukocytes, band neutrophils, and metamyelocytes), and an increased tendency to neutropenia associated with a depleted NSP during experimental sepsis. These observations suggested that new methods of treatment, which induce an increase in the neutrophil supply, might be beneficial for newborn infants with bacterial sepsis.

We previously showed that there is far less granulocyte colony-stimulating factor (G-CSF) production by cells of preterm infants than term infants,<sup>12-15</sup> and a failure to increase G-CSF mRNA expression after infection in newborn animals.<sup>16</sup> We also observed that administration of a single dose of recombinant human (rh)G-CSF to 1-day old newborn rats induced significant neutrophilia,<sup>17,18</sup> whereas administration for 7 days induced neutrophilia and increased the bone marrow neutrophil storage and progenitor pools.<sup>19</sup> Neonatal rats inoculated with lethal doses of group B streptococcus (GBS) and a single pulse of G-CSF had a significantly higher survival rate compared with those treated with antibiotics

alone.<sup>19,20</sup> However, no information exists regarding the toxicity and efficacy of G-CSF administration in infected human infants. Therefore, the safety, pharmacokinetics, and biological efficacy of G-CSF were investigated in a group of 42 newborn infants with presumed sepsis.

### MATERIALS AND METHODS

*Patients.* From July 1, 1991 to March 18, 1993 infants less than 72 hours old with a diagnosis of presumed sepsis at the Children's Hospital of Orange County, Orange, CA, and at the University of Utah Medical Center, Salt Lake City, UT, were eligible for study. Presumed sepsis was defined as having blood cultures obtained and antibiotic therapy initiated. An Investigational New Drug application

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to study rhG-CSF in newborns was obtained from the Food and Drug Administration (IND no. 4112). This protocol was approved by the Human Subjects Review Committees at both institutions and informed consent was obtained before study entry. Infants 26 to 41 weeks gestation, <3 days of age, weighing >800 g, 3rd to 97th percentile for height, weight, and head circumference, with an APGAR score of >5 at 5 minutes of age were eligible for study. Patients were ineligible if they had received corticosteroids, intravenous gammaglobulin, granulocyte transfusions, or had a lethal genetic disorder.

**G-CSF administration.** The rhG-CSF was supplied by Amgen (Thousand Oaks, CA). The rhG-CSF was diluted with 10 cc of 5% glucose in water and 0.2% albumin (2 mg/mL) and infused by pump over 1 hour once daily for 3 consecutive days. Subjects were grouped into three categories: 26 to 30 weeks, 31 to 35 weeks, and 36 to 40 weeks gestation. Three from each group were randomized to receive either placebo (9) or 1.0 (9), 5.0 (9), or 10.0  $\mu\text{g}/\text{kg}/\text{d}$  (9) of rhG-CSF (total of 36 patients). An additional six were randomized to receive either 5.0 or 10.0  $\mu\text{g}/\text{kg}$  every 12 hours for 3 days regardless of gestational age.

**Blood and marrow studies.** Blood was obtained by venipuncture or indwelling catheter. Complete blood counts (CBCs) were performed before and at 2, 6, 24, 48, 72, and 96 hours after rhG-CSF administration. CBCs were performed electronically and differential leukocyte counts were performed manually on Wright-stained blood films. All white blood cell counts were corrected for the presence of nucleated RBCs. Serum chemistries including creatinine, blood urea nitrogen (BUN), electrolytes, total bilirubin, and aspartate aminotransferase (AST) levels were determined immediately before and at 72 hours after the administration of rhG-CSF or placebo.

All patients underwent tibial bone marrow aspiration 72 hours after beginning the rhG-CSF or placebo administration. Differential cell counts (250 to 500 cells) were performed on Wright-stained marrow smears. The proportion of nucleated marrow cells identified as myeloblasts, promyelocytes, or myelocytes were termed the neutrophil proliferative pool (NPP) percent, and the proportion of nucleated marrow cells identified as metamyelocytes, band neutrophils, or segmented neutrophils were termed the NSP percent.

**Hematopoietic progenitors.** Cultures of mature CFU-GM and pluripotent progenitors (CFU-GEMM) were performed at one single laboratory according to a modification<sup>21</sup> of the method of Iscove et al.<sup>22</sup> Light density cells were plated at a concentration of  $5 \times 10^4$  per milliliter. Erythropoietin (2.0 U/mL) and G-CSF, macrophage CSF, granulocyte-macrophage CSF, and interleukin-3 (all at 5  $\mu\text{g}/\text{mL}$ ) were added to the plates, and the plates were examined after 14 days. All cultures were run in quadruplicate. Colonies were enumerated in situ with an inverted, phase-contrast microscope and categorized as previously described.<sup>23</sup>

**Leukocyte C3bi expression analysis.** C3bi expression was determined at one single laboratory at 0 and 24 hours after rhG-CSF or placebo administration. Blood was collected in EDTA-containing tubes and immediately placed on ice. Erythrocytes were removed by density sedimentation using dextran-70 and Hanks' Balanced Salt Solution without calcium or magnesium (Sigma, St Louis, MO). The leukocyte-enriched fraction was removed and a cell count was performed. There were  $1.5 \times 10^6$  cells removed and washed in Dulbecco's phosphate-buffered saline supplemented with azide (0.1%) and bovine albumin (2%). Cells were labeled for fluorescence-activated cell sorter analysis with Leu 15 (C3bi) fluorescein isothiocyanate (FITC)-conjugated monoclonal antibody. Null controls (unstained cells) and isotypic controls (goat anti-mouse IgG<sub>2a</sub> conjugated to FITC) were included at each time point. Samples were incubated, washed, and preserved with 0.05% paraformaldehyde before analysis. Analysis was performed on a Becton Dickinson

(Mountain View, CA) FacStar with gating on the granulocyte population using forward and side scatter parameters. C3bi results are expressed as the percent of hour zero levels.

**G-CSF pharmacokinetics.** Serum for G-CSF quantification was obtained before and at 2, 6, 12, and 24 hours after the start of the first 1-hour infusion in the every 24 hours rhG-CSF dosing group and additionally at 14, 16, 18, and 36 hours for the every 12 hours dosing group. Serum samples were stored at  $-20^\circ\text{C}$  until analyzed. Serum G-CSF concentrations were measured by a sandwich enzyme-linked immunosorbent assay as follows: microtiter plates were coated with polyclonal rabbit anti-rhG-CSF Ig. The rhG-CSF standards, positive controls, and test samples were added and incubated at  $37^\circ\text{C}$  overnight. Horseradish peroxidase-conjugated mouse anti-G-CSF monoclonal antibody (MoAb) was added and incubated for 2 hours at  $37^\circ\text{C}$ . Plates were washed and tetramethylbenzidine added as the substrate. The reaction was stopped after 30 minutes by addition of sulfuric acid (0.5 N). Optical density of the samples was measured at 450 nm with a BioRad (Richmond, CA) enzyme immunoassay reader. Sensitivity of the assay ranged from 50 to 5,000 pg/mL of G-CSF. Values above 5,000 pg/mL were measured by serial dilution of samples. Various concentrations of rhG-CSF (0 to 5,000 pg/mL) were used for the standard curve. All samples were run in duplicate, and data expressed as the mean plus/minus of the standard error of the mean (SEM). Pharmacokinetics were determined by linear regression analysis using the natural logarithm of the serum concentration versus time to determine the half-life ( $T_{1/2} = -0.693/\text{slope}$ ). Correlation coefficients ranged from 0.88 to 0.99.

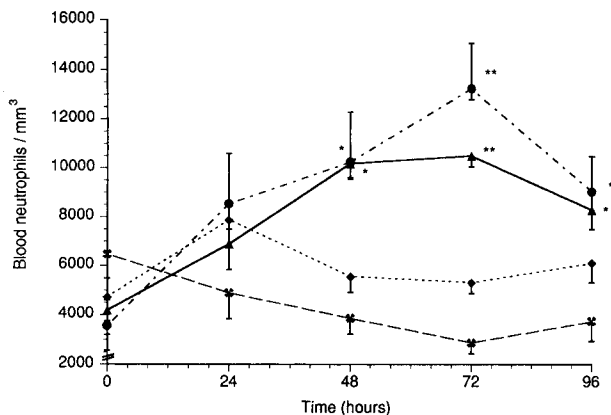
**Statistical analysis.** Statistical analysis was performed using the one-way analysis of variance followed by Dunnett Multiple Comparisons or the Kruskal-Wallis nonparametric analysis of variance (In-Stat statistical program, Graph Pad Software, San Diego, CA). Values are expressed as mean  $\pm$  SEM, and *P* values <.05 were considered significant.

## RESULTS

Of the 42 patients with presumed sepsis, 29 were male and 13 female. Thirty-one required ventilatory support, seven had documented infections, and three had radiographic evidence of pneumonia. One had simultaneous bacteremia with *Staphylococcus epidermidis* and *Klebsiella pneumoniae*, another had GBS bacteremia. Three patients had positive urine cultures: enterococcus (2), *Escherichia coli* (1), two patients were urine antigen reactive for GBS, one had gram-negative rods on Gram stain.

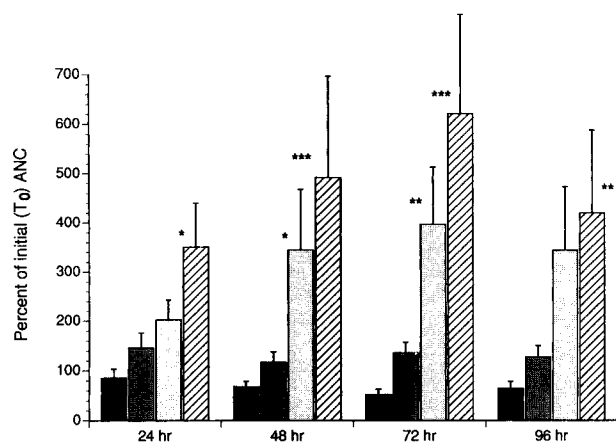
There was no observed toxicity from G-CSF in the patients studied. Specifically, there was no evidence of pulmonary, renal, cardiac, neurologic, gastrointestinal, hepatic, or hematologic toxicity. There was no increase in pulmonary toxicity in those patients requiring mechanical ventilation before G-CSF administration. There were no specific changes in electrolytes, BUN, creatinine, bilirubin, or AST levels. Additionally, there was no evidence of discomfort during the G-CSF infusion. There were no deaths during the study or during the follow-up period (median follow-up time 15 months). Two patients had thrombocytopenia (platelet counts <50,000/mm<sup>3</sup>), which was not exacerbated by rhG-CSF administration. Two patients had neutropenia at the beginning of study (absolute neutrophil count [ANC] <1,000/mm<sup>3</sup>), which resolved with G-CSF administration.

A significant increase in ANC was observed 48, 72, and 96 hours after administration of both 5  $\mu\text{g}/\text{kg}$  and 10  $\mu\text{g}/\text{kg}$

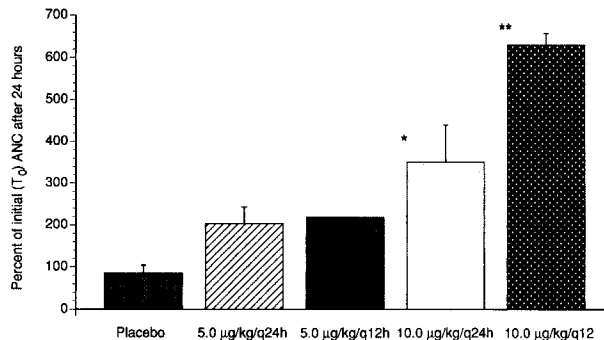


**Fig 1. Blood neutrophil count before and after rhG-CSF or placebo administration.** Doses were given at 0, 24, and 48 hours to 36 infants with presumed sepsis. \**P* < .05, 5.0 µg/kg and 10.0 µg/kg v placebo at 48 and 96 hours; \*\**P* < .01, 5.0 µg/kg and 10.0 µg/kg v placebo at 72 hours. (---\*) Placebo, (---◆) 1.0 µg/kg, (—▲) 5.0 µg/kg, (---●) 10.0 µg/kg.

rhG-CSF (Fig 1). When the ANC was normalized for each patient's hour zero concentration, a significant increase was seen 24 hours after administration of the 10 µg/kg every 24 hours dose (351% ± 89%) (*P* < .05). After 48 hours, a significant increase was observed with 5 and 10 µg/kg every 24 hours dose (345% ± 123%) (*P* < .05) and (492% ± 205%) (*P* < .001). The maximal increase in ANC occurred 72 hours after the 5 and 10 µg/kg every 24 hours dose (397% ± 116%) (*P* < .01) and (621% ± 200%) (*P* < .001), respectively. Neutrophilia was sustained at 96 hours (48 hours after the last dose of G-CSF) with the 10 µg/kg every 24 hours dose (420% ± 168%) (*P* < .01) (Fig 2). Gestational



**Fig 2. Normalized ANC response (percent hour zero ANC) (n = 36) during administration of intravenous rhG-CSF in three doses, one every 24 hours, in newborns with presumed sepsis.** \**P* < .05, 10.0 µg/kg (24 hr), 5.0 µg/kg (48 hr); \*\**P* < .01, 5.0 µg/kg (72 hours), 10.0 µg/kg (96 hours); \*\*\**P* < .001, 10.0 µg/kg (48 hours), 10.0 µg/kg (72 hours) (all compared with placebo). (■) Placebo, (□) 1.0 µg/kg every 24 hours, (▨) 5.0 µg/kg every 24 hours, (▩) 10.0 µg/kg every 24 hours.

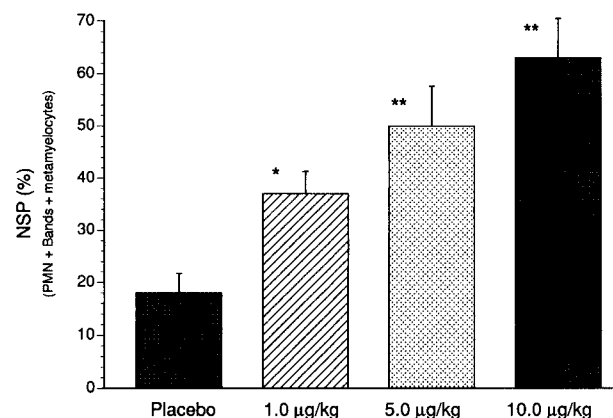


**Fig 3. Percent ANC increase during administration of intravenous rhG-CSF in newborns with presumed sepsis: three doses, one every 12 hours, v three doses, one every 24 hours.** \**P* < .05, 10.0 µg/kg every 24 hours; \*\**P* < .01, 5.0 µg/kg every 12 hours, 10.0 µg/kg every 12 hours (all compared with placebo).

age did not seem to affect the neutrophil response to rhG-CSF.

We observed no difference in ANC 24 hours after the every 12 hours versus every 24 hours dosing of rhG-CSF at 10 µg/kg (629% ± 286% v 351% ± 89%) (*P* = not significant). However, both doses showed a significant increase when compared with placebo (629% ± 286% v 89% ± 19%) (*P* < .05); (351% ± 89% v 85% ± 19%) (*P* < .01). The percent change in ANC at 24 hours with 5 µg/kg every 12 hours was similar to that seen with the 5 µg/kg every 24 hours or 10 µg/kg every 24 hours (218% ± 20% v 203% ± 40% v 351% ± 89%) doses (*P* = not significant) (Fig 3).

Bone marrow aspirates after 72 hours showed a significant dose-dependent increase in the NSP percent (Fig 4). However, there was no increase in the NPP percentage when compared with placebo (13.0% ± 1.8%), 1.0 µg/kg (13.4% ± 3.8%), 5.0 µg/kg (13.5% ± 2.9%), or 10.0 µg/kg every



**Fig 4. Bone marrow NSP response to rhG-CSF.** Tibial bone marrow was obtained 72 hours after administration every 24 hours of intravenous rhG-CSF. Differentials in each group were performed on Wright-stained marrow smears. NSP was defined as the number of nucleated marrow cells identified as metamyelocytes, bands, and segmented neutrophils. \**P* < .05, 1.0 µg/kg v placebo; \*\**P* < .01, 5.0 and 10.0 µg/kg v placebo.

24 hours ( $11.1\% \pm 2.7\%$ ) ( $P =$  not significant). An increase in the CFU-GM + CFU-GEMM was seen at all doses tested compared with placebo ( $53.5\% \pm 8.6\%$ ),  $1.0 \mu\text{g/kg}$  ( $59\% \pm 7.5\%$ ),  $5.0 \mu\text{g/kg}$  ( $71\% \pm 27\%$ ), or  $10.0 \mu\text{g/kg}$  ( $87\% \pm 15\%$ ) (colonies/ $10^4$  cells per plate) ( $P =$  not significant). There was no effect on burst-forming unit-erythrocyte colony formation.

Neutrophil C3bi expression at 24 hours increased after one dose of rhG-CSF when compared with placebo ( $84\% \pm 26\%$ ),  $1.0 \mu\text{g/kg}$  ( $96.8\% \pm 13\%$ ), and  $5.0 \mu\text{g/kg}$  ( $154\% \pm 69\%$ ) ( $P = .25$ ). C3bi expression was significantly increased at 24 hours after the  $10.0 \mu\text{g/kg}$  every 24 hours dose of rhG-CSF ( $233\% \pm 81\% \nu 83\% \pm 26\%$ ) ( $P = .012$ ) (Fig 5).

Peak serum G-CSF levels occurred at 2 hours and were dose dependent: placebo ( $180 \pm 60 \text{ pg/mL}$ ),  $1.0 \mu\text{g/kg}$  ( $2,040 \pm 1,340 \text{ pg/mL}$ ) ( $P < .01$ ),  $5.0 \mu\text{g/kg}$  ( $20,000 \pm 6,260 \text{ pg/mL}$ ) ( $P < .013$ ), and  $10 \mu\text{g/kg}$  every 24 hours ( $126,750 \pm 22,570 \text{ pg/mL}$ ) ( $P < .01$ ). With the addition of a second dose at 12 hours, the patients receiving  $5 \mu\text{g/kg}$  every 12 hours increased their hour 14 serum G-CSF level from  $\leq 900 \text{ pg/mL}$  to  $\geq 50,000 \text{ pg/mL}$ , and the hour 24 level from  $\leq 200 \text{ pg/mL}$  to  $\geq 6,000 \text{ pg/mL}$ . Similarly, the addition of the second dose of  $10 \mu\text{g/kg}$  every 12 hours increased the hour 14 serum G-CSF level from  $\leq 10,000 \text{ pg/mL}$  to  $\geq 70,000 \text{ pg/mL}$  and the hour 24 level from  $\leq 3,000 \text{ pg/mL}$  to  $\geq 13,000 \text{ pg/mL}$ . The hour 36 serum G-CSF level after the every 12 hours dosing remained relatively constant compared with the hour 24 level ( $5.0 \mu\text{g/kg}$  every 12 hours) ( $6,200 \nu 5,000 \text{ pg/mL}$ , 24 hours  $\nu$  36 hours); ( $10.0 \mu\text{g/kg}$  every 12 hours) ( $13,613 \pm 12,887 \nu 10,250 \pm 530 \text{ pg/mL}$ , 24 hours  $\nu$  36 hours) ( $T_{1/2} 4.44 \pm 0.4 \text{ hours}$ ) (Fig 6).

DISCUSSION

An immaturity in quantitative and qualitative aspects of phagocytic immunity contributes significantly to a state of relative immunodeficiency in newborn infants.<sup>24</sup> The incidence of neonatal sepsis increases dramatically with the degree of prematurity, predisposing maternal conditions, and extent of life support procedures required.<sup>1</sup> The risk of bacterial sepsis approaches 25% to 30% in neonates weighing between 500 and 1,000 g at birth.<sup>25</sup>

In the human newborn infant, neutropenia is a common

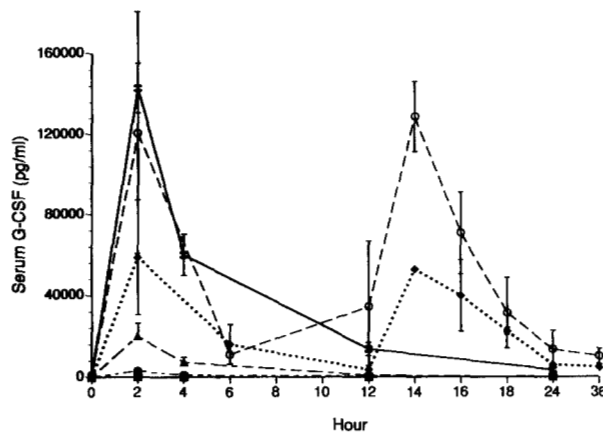


Fig 6. Serum G-CSF concentrations during administration of rhG-CSF in doses every 12 hours and doses every 24 hours. Blood samples were collected 0 and 36 hours. (—■—) Placebo, (—▲—)  $5.0 \mu\text{g/kg}$  every 24 hours, (—◆—)  $5.0 \mu\text{g/kg}$  every 12 hours, (—×—)  $10.0 \mu\text{g/kg}$  every 24 hours, (—○—)  $10.0 \mu\text{g/kg}$  every 12 hours.

finding during bacterial sepsis and is associated with a poor prognosis.<sup>4-7,26</sup> The incidence of bone marrow NSP depletion during neonatal bacterial sepsis ranges between 6% and 62% and is associated with a high mortality rate.<sup>27-30</sup> Decreased myeloid progenitor cell pools and reduced total body neutrophil storage pools seem to predispose newborn rats to depletion of their supply of neutrophils during bacterial sepsis.<sup>8,9,31</sup> Recently, adult donor neutrophil transfusions have been successfully used to improve the outcome in the treatment of newborn infants with neutropenia and bacterial sepsis.<sup>32,33</sup>

G-CSF is a physiologic regulator of myelopoiesis and an activator of mature effector neutrophil function.<sup>34</sup> It supports the clonal growth of neutrophil progenitors,<sup>35</sup> primes neutrophils to increased expression of chemotactic receptors, enhances bacteriocidal phagocytic activity and superoxide generation, and also enhances antibody-dependent cellular cytotoxicity.<sup>36-38</sup> Basal serum concentrations of G-CSF are generally in the picogram per milliliter range.<sup>39</sup> However, during states of increased demand such as during bacterial

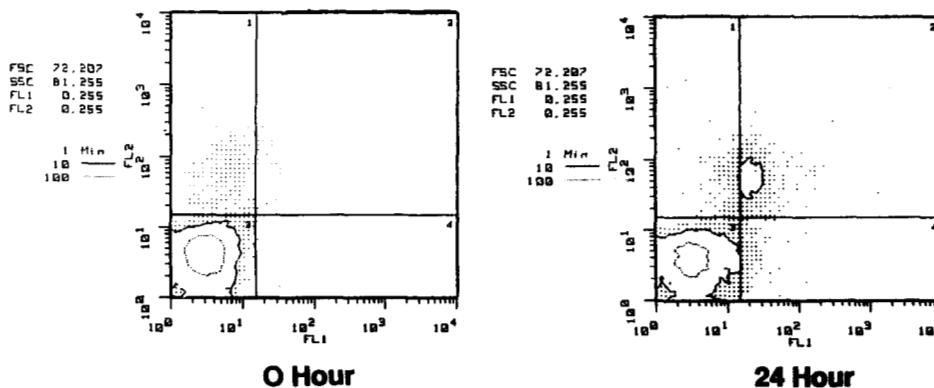


Fig 5. A representative histogram of C3bi expression determined before and 24 hours after rhG-CSF administration. Null controls and isotypic controls were included at each time point. Analysis was performed by gating on the neutrophil population using forward and side scatter parameters, and comparing the intensity of fluorescent staining at various time points.  $P < .01$ ,  $10.0 \mu\text{g/kg}$  every 24 hours  $\nu$  placebo.



sepsis, G-CSF concentrations increase significantly, often into the nanogram per milliliter range.<sup>40,41</sup> During severe neutropenia after myeloablative therapy, an inverse correlation has been shown between serum G-CSF concentrations and ANC.<sup>42</sup>

Compared with adults, newborn infants do not seem to generate G-CSF effectively. Analysis of G-CSF mRNA expression from cord and adult mononuclear cells by Northern blot hybridization has shown negligible basal G-CSF expression.<sup>13</sup> Similarly, after activation of cord mononuclear cells, there is significantly less G-CSF protein and mRNA expression compared with similarly activated adult mononuclear cells.<sup>13</sup> Even lower expression is observed using mononuclear cells of preterm neonates.<sup>14</sup> Furthermore, G-CSF serum concentrations from term newborn infants with neutropenia are significantly lower than in adults with similar degrees of neutropenia.<sup>14</sup> Finally, during experimental sepsis, newborn rats fail to increase G-CSF mRNA expression from bone marrow, lung, liver, and spleen.<sup>16</sup>

G-CSF has been shown to enhance host defense during experimental sepsis in adult animals.<sup>43-48</sup> We previously showed that a single dose of 5.0  $\mu\text{g}/\text{kg}$  of rhG-CSF to newborn rats results in a significant threefold increase in neutrophilia that is sustained 24 hours after administration.<sup>20</sup> The effects of seven consecutive daily doses of 5.0  $\mu\text{g}/\text{kg}/\text{d}$  of rhG-CSF to newborn rats induces a significant fivefold to sixfold increase in ANC and a twofold increase in bone marrow NSP.<sup>19</sup> Single and 7-day administration of rhG-CSF in newborn rats also results in a significant reduction in the mortality rate during experimental GBS infection.<sup>19,20</sup>

Recombinant human G-CSF has been successfully used in a number of clinical conditions, including hematologic and oncologic disorders and immunodeficiency syndromes. It has been shown to reverse cyclic neutropenia, induce neutrophilia in children with congenital agranulocytosis (Kostman's syndrome) and severe chronic neutropenia, and to induce neutrophilia in bone marrow failure syndromes such as aplastic anemia.<sup>49-52</sup> It has also been successfully used to reduce the period of neutropenia, fever, and sepsis after administration of multiagent chemotherapy for acute leukemias and solid tumors.<sup>53-55</sup> Lastly, rhG-CSF hastens myeloid recovery after myeloablative therapy in bone marrow transplantation and reduces neutropenia in patients receiving myelosuppressive therapy for acquired immunodeficiency syndrome.<sup>43</sup>

The use of G-CSF in neonates with presumed sepsis seemed to be safe and well tolerated in the present study. We determined that the pharmacokinetics of intravenous rhG-CSF in neonates were first order, with a half-life of 4.4 hours. Peak serum concentrations occurred within 2 hours and were dose dependent. Older children receiving 5.0  $\mu\text{g}/\text{kg}$  rhG-CSF subcutaneously after chemotherapy achieve peak levels of 20,000 pg/mL, comparable to the levels achieved by newborn infants in the present study.<sup>56</sup> In adults treated with rhG-CSF intravenously after bone marrow transplantation, peak G-CSF concentrations also approach 20,000 pg/mL.<sup>55</sup>

In the neonates we studied, G-CSF induced a significant

increase in the ANC and resulted in a twofold to threefold increase in the NSP. This corresponds with the twofold increase in NSP we observed after 7 days of rhG-CSF administration to newborn rats. As was the case in the neonatal rats, we also saw no significant change in the NPP. Additionally, rhG-CSF (10.0  $\mu\text{g}/\text{kg}/\text{d}$ ) induced a significant increase in neutrophil C3bi expression. The enhancement of neonatal polymorphonuclear (PMN) C3bi expression by rhG-CSF in this study suggests that rhG-CSF may induce functional activation of neonatal neutrophils. Increased PMN C3bi expression could result in enhancement of PMN adherence and/or aggregation, leading to either improved function and/or increased toxicity.

The high incidence of neutropenia and mortality during bacterial sepsis in the neonate may, in part, be secondary to deficient G-CSF production. We have shown that administration of rhG-CSF to newborn infants with presumed sepsis was well tolerated and induced neutrophilia and an increase in NSP. On this basis, we speculate that future strategies for the treatment of neonates with overwhelming bacterial sepsis and neutropenia might benefit from the adjuvant use of rhG-CSF. A multicenter, phase II/III, randomized, prospective, double-blind, placebo-controlled trial is required to determine the efficacy of rhG-CSF in this clinical setting.

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