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Modulation of Neonatal Myelopoiesis in Newborn Rats after 7 Days' Administration of Either Granulocyte-Monocyte Colony Stimulating Factor or Interleukin-3[1]

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ABSTRACT. Single-pulse administration of either recombinant human granulocyte-monocyte colony stimulating factor or recombinant human granulocyte colony stimulating factor to newborn rats has previously been demonstrated to increase the peripheral neutrophil count and modulate bone marrow (BM) neutrophil pools. In our present study, we investigated the effects of 7 d of either recombinant murine granulocyte-monocyte colony stimulating factor (rmGM-CSF) (75 μg/kg/d) or recombinant murine IL-3 (rm IL-3) (10 μg/kg/d) on newborn rat myelopoiesis. Sprague Dawley newborn rats (≥24 h) were injected (intraperitoneally) daily for 7 d with either rmGM-CSF, rmIL-3, or PBS/BSA. rmGM-CSF induced a significant increase in the peripheral neutrophil count on d 3 ($p < 0.03$) and d 7 ($p < 0.001$) (75% increase). Additionally, rmGM-CSF induced a 50% increase in the BM neutrophil storage pool ($p < 0.025$). rmIL-3 increased the BM colony forming unit-granulocyte monocyte pool ($p < 0.001$); however, it failed to increase the peripheral neutrophil count or BM neutrophil storage pool. Neither CSF increased the BM neutrophil proliferative pool or BM colony forming unit-granulocyte monocyte proliferative rate. Additionally, 7 d of rmGM-CSF with or without antibiotics did not synergistically alter the mortality rate after group B streptococcol inoculation. This study suggests that rmIL-3 appears to stimulate more neonatal myeloid committed progenitor cell activity compared with rmGM-CSF. Optimal modulation of neonatal myelopoiesis may require the use of a sequential combination of hematopoietic CSF, namely an early-acting CSF followed by a more lineage myeloid-specific CSF. (*Pediatr Res* 29: 504–509, 1991)

Abbreviations

CSF, colony stimulating factor
G-CSF, granulocyte colony stimulating factor
GM-CSF, granulocyte-monocyte colony stimulating factor
NSP, neutrophil storage pool
NPP, neutrophil proliferative pool
CFU-GM, colony forming unit-granulocyte monocyte
GBS, group B streptococcus
rh, recombinant human
rm, recombinant murine
i.p., intraperitoneal(ly)

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Neonatal host defense is characterized by a significant developmental immaturity in various components of the immune system, predisposing the neonate to a high mortality rate during overwhelming bacterial infection (1). Significant reductions in myeloid progenitor proliferation and bone marrow NSP, and, additionally, a tendency to develop peripheral neutropenia, characterize some of the most important deficiencies in neonatal host defense (2–4). During experimental sepsis, newborn rats also demonstrate markedly different myeloproliferative responses compared with their adult counterparts (5). Newborn rats, inoculated with bacterial organisms such as GBS fail to increase their already high myeloid progenitor proliferative rate, decrease their myeloid bone marrow committed stem cell pool (CFU-GM), and, most importantly, deplete their already reduced NSP (polymorphonuclear leukocyte + band + metamyelocyte) (5).

Bone marrow myeloid proliferation and egress of mature effector neutrophils are influenced in part by the proliferative and differentiating effects of hematopoietic CSF (6–9). Some CSF induce proliferation of stem cells, whereas others affect more lineage-specific, later-acting, committed progenitor cells and the functional activation of mature effector cells. Class 1 factors such as GM-CSF and IL-3 appear to act on earlier and more pluripotent stem cell progenitors compared with class 2 factors such as G-CSF, which have been demonstrated to act on more lineage-specific, committed myeloid progenitor cells (6–9). CSF appears, in fact, to be the major regulator of increased peripheral myeloid cell production during states of sepsis or increased demand (6).

IL-3 was first isolated from the Gibbon cell line MLA-144 and has recently been demonstrated to be present in the growth factor-rich chromosome 5q region (10–13). Human IL-3 has a limited homology at the amino acid level with murine IL-3 (29%) (6). IL-3 appears to stimulate the growth and proliferation of very early pluripotent bone marrow progenitor cells and, in adult studies of hematopoiesis, has been demonstrated to stimulate the clonal proliferation of erythroid, myeloid, and megakaryocytic bone marrow progenitor cells (14, 15). IL-3 appears to preferentially induce the stimulation of early progenitor cells. Effects of IL-3 on later, more lineage specific, committed progenitor cells may be secondary to the effects of class 2 CSF such as G-CSF, monocyte-CSF, and erythropoietin.

GM-CSF, another lymphokine, was first purified to homogeneity from the Mo T lymphoblastoid cell line (16). Human GM-

CSF is also a small glycoprotein (22 000 D) and, despite sharing a 60% amino acid homology with murine GM-CSF, appears to have diminished myeloproliferative effects when used in experiments with murine animals (8). GM-CSF also seems to modulate proliferation of bone marrow progenitor cells, but has been demonstrated to influence the growth of only CFU-GM and CFU-eosinophil, resulting in an increase in peripheral granulocytes, monocytes, and eosinophils (17). GM-CSF, however, besides having the ability to induce proliferation of committed progenitor cells, has also been demonstrated to enhance mature effector neutrophil function of both neonatal and adult mature neutrophils (18).

We have previously demonstrated that single-pulse administration of G-CSF or GM-CSF to newborn rats results in a significant increase in peripheral neutrophilia (19). Additionally, we have demonstrated that single-pulse rhG-CSF given prophylactically or simultaneously with antibiotics during experimental GBS infection (in newborn rats) reduces the mortality rate compared with antibiotic therapy alone (20). Recently, we have demonstrated that prolonged prophylactic administration (7 d) of a more lineage-specific CSF such as rhG-CSF to newborn rats significantly modulates myelopoiesis and the response to GBS sepsis (21). In our present study, we have investigated and compared the effects of 7 d of administration of both class 1 factors, rmGM-CSF and rmIL-3, and their modulation of myelopoiesis in the newborn rat.

MATERIALS AND METHODS

Murine GM-CSF. rmGM-CSF (Immunex Corporation, Seattle, WA) is expressed in yeast, and purified to homogeneity before formulation in RPMI. SDS-PAGE was used to confirm the purity of the product by the presence of a single protein band. Biologic activity of 4×10^7 U/mg was determined by granulocyte macrophage colony formation (CFU-GM) of nonadherent mouse bone marrow cells in semisolid media. The Limulus amebocyte lysate assay was used to demonstrate the absence of measurable endotoxin. Purified rmGM-CSF was used at concentrations of 75.0 μg/kg (diluted with PBS, pH 7.4, and 0.025% BSA; Sigma Chemical Co., St. Louis, MO). A dose-response study of 1–75 μg/kg/d demonstrated maximal neutrophil counts with 75 μg/kg/d.

Murine IL-3. Mouse recombinant IL-3 (Genzyme, Cambridge, MA), is expressed in COS cells and purified to homogeneity before formulation in PBS/0.1% BSA. SDS-PAGE was used to confirm the purity of the product by the presence of a single protein band. The biologic activity was determined to be 1×10^6 U/mg, and the Limulus amebocyte lysate assay was used to demonstrate the absence of measurable endotoxin. rmIL-3 was used at concentrations of 10 μg/kg (diluted with PBS and 0.025% BSA). A dose-response study of 0.1–10.0 μg/kg/d demonstrated maximal neutrophil counts with 10 μg/kg/d.

GBS. GBS, type III, Norris, was kindly provided by Dr. Gerald Fisher (Uniformed Services Hospital, Bethesda, MD). The organism was isolated from an infected neonate and serotyped by the precipitin method using rabbit antisera. The organism was grown in Todd-Hewitt broth to logarithmic phase and then aliquoted and stored at −70°C until use. Aliquots were thawed and allowed to grow to maximum phase growth in fresh Todd-Hewitt broth. Organisms were then sedimented by centrifugation and washed three times in sterile PBS. Concentration of bacteria was standardized by its OD at 620 nm, and a suspension of 3×10^6 organisms/g body wt/0.100 mL was prepared for injection.

Animal inoculation. Litters of albino Sprague Dawley neonatal rats (Bantin-Kingman Laboratories, Fremont, CA) ≤24 h old (6–8 g) were used during this study. Mothers of the litters were received 1 wk before delivery and were housed at the vivarium at the University of California Irvine Medical Center. They were maintained at constant room temperature, with water and rodent feed (Purina Chow, Ralston-Purina Co., St. Louis, MO) *ad libitum*. Approval for this study was granted by the Animal Use Committee at the university. Before inoculation of the neonatal rat, the site of injection was washed with Betadine solution (povidone-iodine, 10%; Purdue Frederick, Norwalk, CT). Daily (7 d) i.p. injections of rmGM-CSF or rmIL-3 were accomplished with a sterile tuberculin syringe fitted with a 27.5-gauge needle. GBS was injected s.c. at the tail region of the animal with a sterile tuberculin syringe; antibiotics were administered intramuscularly at the hind leg.

Quantification of circulating, proliferative, and storage pools. Blood samples were obtained daily by nicking the jugular vein with a sterile needle and collecting 10 μL of free-flowing blood. Samples were electronically counted (Serano-Baker Diagnostics, Allentown, PA); blood smears were prepared and stained with Wright stain, and a 100–200 cell differential was performed. Absolute neutrophil counts were determined by the multiplication of the nucleated cell count by the percentage of neutrophils in the differentials. Bone marrow NSP (polymorphonuclear leukocyte + band + metamyelocyte) and NPP (blasts + promyelocyte + myelocyte) were determined on d 8 by the method of Christensen *et al.* (5). Briefly, neonatal femurs were aseptically removed, and the contents flushed into a known quantity of Hanks' balanced salt solution (Gibco Laboratories, Grand Island, NY). Livers and spleens were also removed on d 8 in a similar manner, and finely minced in a known quantity of Hanks' balanced salt solution. Electronic cell counts were performed on the bone marrow, and a 500-cell differential count was obtained on Wright-stained cytospin preparations.

CFU-GM proliferative rate. Proliferative rates of CFU-GM were evaluated by the thymidine suicide method of Christensen *et al.* (5). Bone marrow and liver and spleen cells from neonatal rats were placed into each of two 50-mL centrifuge tubes. To the first aliquot was added 0.34 μg nonradioactive thymidine in a volume of 0.1 mL. The other aliquot received 0.34 μg methyl-^{3}H-thymidine containing 0.1 mCi (sp act, 75 Ci/mmol; ICN Radiochemicals, Irvine, CA). The tubes were incubated for 20 min at 37°C while agitating every 5 min. Thymidine uptake was halted by adding 30 mL of ice cold α-minimum essential medium with 5% FCS and 100 μg/mL nonradioactive thymidine. The cell suspensions were then centrifuged and washed twice with the α-minimum essential medium FCS/thymidine media. Cells were then added in the same 1.1% methylcellulose/α-media described above. Colonies were allowed to develop for 14 d in a 5% CO_2 incubator at 37°C, and thymidine suicide rate was determined by subtracting the average number of colonies formed per plate by cells exposed to [^{3}H]thymidine from the average number of colonies per plate formed by cells exposed to nonradioactive thymidine, and dividing by the average colonies per plate from cells exposed to nonradioactive thymidine.

CFU-GM colony quantification. Bone marrow and liver/spleen cells were collected as described above. Cells were then suspended in α-media (Gibco Laboratories) with 1.1% methylcellulose (Terry Fox Laboratories, Vancouver, BC), 30% vol/vol FCS (Hyclone Laboratories, Logan, UT), 10% BSA (Sigma Chemical Co.), 14.3×10^{-3} M mercaptoethanol, 0.01% vol/vol murine spleen cell conditioned medium (PWM-SCCM; Terry Fox Laboratories), and 1 U/mL erythropoietin (Amgen, Thousand Oaks, CA). Penicillin (100 000 U/L) and streptomycin (100 mg/L) were also added. Cell suspensions were plated in triplicate in 10 × 35 mm tissue culture dishes (Nunc, Copenhagen, Denmark) and incubated at 5% CO_2, 37°C, in a high humidity atmosphere. Cultures were evaluated at 14 d with aggregates of >50 cells considered "colonies." Colonies were plucked at random, placed on a slide and stained, and lineage specificity was confirmed.

Treatments. To determine rmGM-CSF effect on the modulation of sepsis, newborn litters were given rmGM-CSF i.p. (75.0 μg/kg) or PBS/0.025% BSA (control group) for 7 d. Litters were given several forms of therapy, and were inoculated with GBS (1×10^8 organisms/kg) s.c. on d 8. Treatment modalities included the following: *1*) 7 d rmGM-CSF i.p. 75 μg/kg/d; *2*) 7 d of PBS/

0.025% BSA; *3*) 7 d rmGM-CSF and antibiotics, ampicillin (Bristol Laboratories, Evansville, IN) (150 mg/kg/d), and gentamicin (Elkins-Sinn, Cherry Hill, NJ) (6.5 mg/kg/d) intramuscularly divided and administered twice a day, started 24 h after GBS; and *4*) 7 d PBS/0.025% BSA and the same antibiotic therapy. Mortality and morbidity were monitored throughout the sepsis study.

Statistical analysis. All results are expressed as mean values plus or minus SEM of 8–15 animals, or three to five replicates of blood, bone marrow, or liver/spleen samples. The probability of significant differences when comparing two treated groups was determined with the use of the unpaired *t* test, and the probability of significant differences when examining multiple treatments was determined by using the analysis of variance followed by the Student-Newman-Keuls multiple range tests to define the unique subsets within the study. Statistical analyses were performed using the Biostat I statistical program (Sigma Soft, Placentia, CA) for the IBM personal computer. p values <0.05 are considered significant.

RESULTS

The absolute neutrophil count, *i.e.* peripheral neutrophilia, was determined on d 1, 3, and 7 after i.p. rmGM-CSF and i.p. rmIL-3 administration. A dose of 10 μg/kg of i.p. rmIL-3 was chosen after studying the dose response of rmIL-3 (0.1, 1.0, and 10.0 μg/kg). Day 7 absolute neutrophil count: 0.1, 541 ± 70 mm^3; 1.0, 540 ± 144 mm^3, and 10.0, 896 ± 148 mm^3 (0.1 and 1.0 *versus* 10.0, $p < 0.04$). Day 8 bone marrow CFU-GM: 0.1, 47 ± 2.9 *versus* 1.0, 46 ± 2.2 *versus* 10.0, 68 ± 2.2; 0.1 and 1.0 versus 10.0, $p < 0.02$. Within the first day of administration, there were no significant differences between rmGM-CSF and rmIL-3 compared with controls. However, on d 3 and additionally after 7 d of administration, there was a significant difference in the peripheral neutrophil count after i.p. rmGM-CSF compared with control treated animals. There was no significant difference, however, after i.p. rmIL-3 administration (Fig. 1). On d 7, the absolute neutrophil count (mm^3) after 7 d of i.p. rmGM-CSF was 2218 ± 54 *versus* 1288 ± 169 ($p < 0.001$). Comparatively, 7 d of i.p. rmIL-3 did not induce a significant increase in peripheral neutrophilia: d 7, 789 ± 193 *versus* 642 ± 282 mm^3; p = NS, rmIL-3 *versus* PBS/BSA. Additionally, rmIL-3 failed to induce any significant changes in the peripheral basophil or eosinophil count and did not result in any change in the peripheral platelet count: d 7, 791 ± 95 *versus* 884 ± 74 × $10^3/mm^3$; p = NS, rmIL-3 *versus* PBS/BSA.

The bone marrow NSP was determined on d 8 after 7 consecutive days of either i.p. rmGM-CSF or i.p. rmIL-3. rmGM-CSF induced a significant increase in the bone marrow NSP (50%) ($p < 0.025$) (Table 1). rmIL-3, however, a more early-acting hematopoietic growth factor, failed to induce a significant increase in the bone marrow NSP (Table 1). The bone marrow NPP was also calculated after 7 d administration of i.p. rmGM-CSF and rmIL-3. There was, however, no significant difference in the bone marrow NPP by either hematopoietic colony stimulating factor (Table 2).

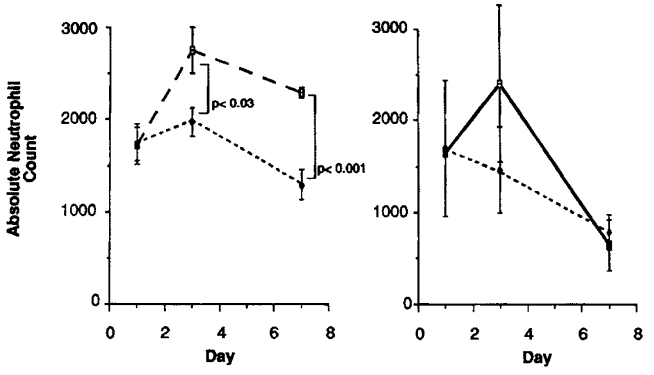

Fig. 1. Neonatal Sprague Dawley rats (≤24 h old) received rmGM-CSF, rmIL-3, or 0.025% PBS/BSA by i.p. injection daily for 7 d. Blood samples were obtained by nicking the jugular vein with a sterile needle and collecting 10 μL of free-flowing blood. Samples were electronically counted, and a 100–200 cell differential was performed on Wright-stained blood smears. Values reflect the mean absolute neutrophil count (mm^3) ± SEM of 20 animals at each time point. rmGM-CSF (— — —), rmIL-3 (____), PBS/0.25% BSA (---).

The bone marrow committed myeloid stem cell pool (CFU-GM) was additionally determined on d 8 after 7 consecutive days of both i.p. rmGM-CSF and i.p. rmIL-3. Although i.p. rmGM-CSF failed to induce a significant increase in myeloid progenitor stem cell colony formation, rmIL-3 induced a mild but significant increase (15%) $p < 0.001$) in myeloid CFU-GM colony formation (Table 1).

Combined liver and spleen NSP, NPP, myeloid CFU-GM colony formation, and CFU-GM proliferative rates were additionally assessed after 7 d administration of both i.p. rmGM-CSF and i.p. rmIL-3. There was, however, no significant increase by either i.p. CSF on combined liver/spleen NSP, NPP, CFU-GM colony formation, or CFU-GM proliferative rate (Table 2).

Eight-d-old newborn rats were inoculated with GBS, and the mortality rate after 7 d of i.p. rmGM-CSF or PBS/BSA was evaluated. Four groups of animals were compared in this part of the study. The first group of animals received 7 d of PBS/BSA and were then inoculated with GBS. The second group of animals received 7 d of i.p. rmGM-CSF and were then subsequently inoculated with GBS. The third group of animals received the identical PBS/BSA as in group 1 and GBS inoculation on d 8, and then subsequently were given ampicillin and gentamicin 24 h after GBS inoculation. The last group of animals received 7 d of rmGM-CSF inoculated with GBS on d 8 and received ampicillin plus gentamicin 24 h after GBS inoculation. Although the group of animals pretreated with 7 d of rmGM-CSF had a mild increase in the peripheral neutrophil count and bone marrow NSP, there was no reduction in the mortality rate with or without antibiotic administration (90 h) (rmGM-CSF + antibiotic *versus* PBS/BSA + antibiotic, 57 *versus* 59% survival rate; p = NS) (Fig. 2).

DISCUSSION

Although the high mortality rate associated with bacterial sepsis during the newborn period appears to be multifactorial, one of the most important deficits of the neonatal immune system contributing to this high incidence is the quantitative deficiency of the myeloid and phagocytic system (1). Future preventive and/or concurrent therapy of neonatal sepsis, therefore, may require the use of adjuvant immunologic therapy. Newborn rats have significantly reduced myeloid progenitor pools (CFU-GM) (20%), and require 4 wk of developmental maturation to attain normal adult levels. Additionally, newborn rats have markedly reduced bone marrow NSP (25%), and also require 4–6 wk to mature to normal adult levels. We therefore have begun to investigate new pharmacologic and biologic methods to modulate newborn myeloid proliferation and differentiation (22–25). Diminished newborn myeloid progenitor cells (CFU-GM), near maximal proliferative capacity of myeloid progenitor cells, and an accelerated release of NSP cells from the newborn bone marrow reserve predispose the newborn to depletion of bone marrow mature neutrophil reserves and the subsequent development of significant peripheral neutropenia during overwhelming bacterial sepsis (2, 4, 26).

Recently, GM-CSF has been demonstrated to enhance myelopoiesis after bone marrow transplantation and has been used as adjuvant therapy in adults receiving intensive chemotherapy and, additionally, as specific immunomodulating therapy in patients with aplastic anemia, myelodysplasic syndromes, and AIDS (9). GM-CSF has been demonstrated to enhance hemato-

Table 1. *Effect of 7 d of i.p. rmGM-CSF or rmIL-3 on newborn rat bone marrow NSP and colony formation**

	rmGM-CSF	PBS/BSA	rmIL-3	PBS/BSA
Bone marrow NSP (mm^3)	3427 ± 238†	2280 ± 165	6964 ± 730	5201 ± 334
Bone marrow CFU-GM colonies	95 ± 9	92 ± 7	123 ± 3†	107 ± 4

* rmGM-CSF, rmIL-3, or 0.025% BSA/PBS was administered to neonatal rats (≤24 h old) by i.p. injection for 7 d. Bone marrow NSP was determined by the aseptic removal of the femurs and the flushing of the bone marrow into a known quantity of Hanks' balanced salt solution. Electronic cell counts were performed, and a 500-cell differential was obtained on Wright-stained cytospin preparations. Cells for colony assays were suspended in 1.1% methyl/cellulose α-media, and triplicate cultures were plated at 2.0×10^5 cells per plate. Colonies were evaluated at d 14 by scoring the aggregates and plucking colonies at random for differential staining. Data reflects the mean value ± SEM of two experiments done in triplicate in which 12 animals per treated litter were analyzed.

† Significance of $p < 0.05$.

Table 2. *Effect of 7 d of i.p. rmGM-CSF or rmIL-3 on newborn rat bone marrow and liver/spleen myelopoiesis**

	rmGM-CSF	PBS/BSA	rmIL-3	PBS/BSA
Bone marrow NPP (mm^3)	2393 ± 88	2336 ± 240	3014 ± 625	3641 ± 440
Bone marrow CFU-GM proliferative rate (%)	57.7 ± 6.1	52.1 ± 9.1	73.2 ± 2.5	69.5 ± 5
Liver/spleen NSP (mm^3)	16402 ± 5886	13218 ± 1105	20083 ± 5765	23785 ± 4519
Liver/spleen NPP (mm^3)	21748 ± 2264	23298 ± 50	36033 ± 17572	23115 ± 1641
Liver/spleen CFU-GM colonies	19 ± 9	18 ± 9	15 ± 11	13 ± 9
Liver/spleen CFU-GM proliferative rate (%)	35.6 ± 9.0	39.6 ± 15	30.2 ± 9.2	39.6 ± 15

* rmGM-CSF, rmIL-3, or 0.025% BSA/PBS was administered to neonatal rats by i.p. injection daily for 7 d. Bone marrow cells were recovered after the aseptic removal of the femurs and flushing the bone marrow into a known quantity of balanced saline. Liver/spleen cells were isolated from the organs, which were aseptically removed and finely minced in a known quantity of saline. Bone marrow and liver/spleen NPP and NSP were determined by performing electronic cell counts, and a 500-cell differential was obtained on the Wright-stained cytospin preparations. Bone marrow and liver/spleen CFU-GM proliferative rates were evaluated by the thymidine suicide method. Bone marrow and liver/spleen CFU-GM were determined by suspending isolated cells in 1.1% methylcellulose/α-media and plating 2.0×10^5 cells in triplicate. Colonies were evaluated at d 14 by scoring the aggregates and plucking colonies at random for differential staining. Values represent the mean value ± SEM of two experiments done in triplicate in which 12 animals per treated litter were analyzed.

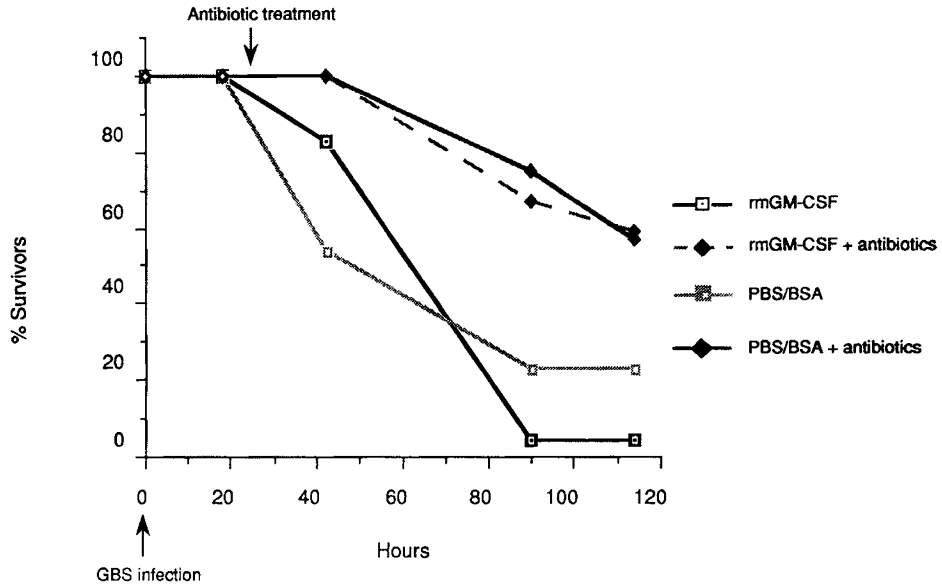

Fig. 2. Litters of neonatal rats (≤24 h old) received rmGM-CSF or 0.025% BSA/PBS i.p. for 7 d. Litters were inoculated with 1×10^8 GBS/g on d 8, and 24 h later, groups received antibiotics (ampicillin, 150 mg/kg/d, and gentamicin, 6.5 mg/kg/d) every 12 h or no additional treatment. The graph represents the percent survival after 96 h.

poiesis and host defense by inducing peripheral neutrophilia secondary to egress of NSP cells within the first 24 h of administration, to stimulate sustained peripheral neutrophilia by inducement of early myeloid progenitor cells to undergo proliferation and differentiation, and, lastly, to correct *in vitro* mature effector neutrophil physiologic dysfunction (27–29). Human IL-3, however, has only recently begun to undergo investigation in human clinical trials, and is just entering phase I and phase II trials in patients with bone marrow failure (30). Additionally, human IL-3, when administered to nonhuman primates, has been demonstrated to possess synergistic activity with GM-CSF in stimulating hematopoiesis and the induction of increased circulating levels of eosinophils, monocytes, and neutrophils (31). Previous primate studies have suggested that the hematologic effect of IL-3 alone of inducing peripheral neutrophilia is markedly reduced compared with the leukocytosis observed after either GM-CSF or G-CSF.

In our present study, we investigated the effects of both rmGM-CSF and rmIL-3 on neonatal myelopoiesis. Our study has suggested that rmGM-CSF, a more lineage-specific, class 1 hematopoietic CSF, induced more peripheral neutrophilia and a 50% increase in the bone marrow NSP, but had little effect on myeloid CFU-GM colony formation, proliferative rate, and bone marrow proliferative pool. In contrast, rmIL-3, an earlier and less lineage-

specific multicolony stimulating factor, failed to induce any peripheral neutrophilia and had no effect on bone marrow NSP, NPP, or bone marrow CFU-GM proliferative rate. rmIL-3, however, appeared to mildly increase bone marrow CFU-GM colony formation. Neither rmGM-CSF nor rmIL-3 had any significant effect on the combined liver/spleen NSP, NPP, CFU-GM colony formation, or CFU-GM proliferative rate.

Previous studies by Erdman *et al.* (4) demonstrated that 1-wk-old neonatal rats possessed only 50% of normal bone marrow storage pools compared with developmentally normal adult rats. In our present study, 7 d of administration of rmGM-CSF to newborn rats appeared to increase the 1-wk-old NSP by 50%. This 50% increase in the NSP approximates the normal values attained in normal adult rat bone marrow NSP. In comparison, in our previous studies using 7 d of rhG-CSF, a more myeloid lineage-specific hematopoietic CSF, we demonstrated a 100% increase in the 1-wk-old newborn rat NSP (21).

Seven d of rmGM-CSF also induced a significant increase in the d 7 peripheral neutrophil count (75%, $p < 0.001$). However, this increase in the d 7 peripheral neutrophil count was somewhat weaker compared with our previous 7-d studies with rhG-CSF, which resulted in an increase in the d 7 peripheral neutrophil count of almost 750% (20, 21). rhIL-3, however, did not induce any increase in the peripheral neutrophil count during the entire 7 d of administration. Our findings are consistent with previous (7-d rhIL-3) studies of primates, which also failed to demonstrate an increase in the peripheral neutrophil count (31–33).

We additionally assessed the effect of 7 d of i.p. rmGM-CSF or i.p. rmIL-3 on d 8 bone marrow CFU-GM colony formation. Although rmGM-CSF had a mild, but not statistically significant increase in bone marrow CFU-GM colony formation, rmIL-3, an earlier-acting hematopoietic CSF, induced a significant increase in bone marrow CFU-GM colony formation. Both rmGM-CSF and rmIL-3 also appeared to mildly increase the bone marrow CFU-GM proliferative rate. Neither rmGM-CSF nor rmIL-3 resulted, however, in an increase in the bone marrow NPP or in the myeloid proliferative or storage pools of the combined liver/spleen homogenates. Our findings in the present newborn rat study are similar to findings of Pojda *et al.* (34), who studied the hematologic effects of daily rmGM-CSF in normal adult BDF mice for a period of 11 wk. Specifically, after daily injections of rmGM-CSF for 1 wk, they found no increase in bone marrow CFU-GM or colony forming unit of mixed erythroid and granulocyte-monocyte colonies formation (34). Additionally, this latter study also failed to demonstrate an increase in spleen CFU-GM progenitor proliferation after 7 d of rmGM-CSF in adult mice (34). Metcalf *et al.* (35) additionally studied the hematologic effects of 6 d of i.p. rmGM-CSF administered to adult BALB/c mice. This study, similar to ours, also demonstrated an increase in the 7-d peripheral neutrophil count but, in contrast to our study, showed a mild decrease in CFU-GM after 6 d of i.p. rmGM-CSF.

Because 7 d of rmGM-CSF induced a mild increase in peripheral neutrophil count and an increase in the bone marrow NSP, we additionally investigated whether this effect would modulate the mortality associated with GBS infection. Seven d of rmGM-CSF administration, however, failed to synergistically enhance survival after GBS experimental infection in antibiotic-treated newborn animals. Both groups of animals receiving PBS/BSA or rmGM-CSF plus antibiotics had a 57–59% survival rate after GBS inoculation. This, however, contrasts with our previous studies with 7 d rhG-CSF, which demonstrated an almost 100% increase in survival over PBS/BSA plus antibiotic-treated animals (20, 21). In the rhG-CSF study, however, 7 d of this class II lineage-specific myeloid growth factor induced an almost 750% increase in the peripheral neutrophil count, and an almost 100% increase in the bone marrow NSP. In our present study, rmGM-CSF only induced a 75% increase in peripheral neutrophil count, and only a 50% increase in the bone marrow NSP. A comparison of these two studies suggests that prophylactic CSF administration requires a very significant increase in the peripheral neutrophil count and bone marrow NSP to prevent significant peripheral neutropenia and bone marrow NSP depletion during experimental sepsis.

It appears that IL-3 alone has minimal effects on the inducement of peripheral neutrophilia, bone marrow, and liver/spleen storage pools. IL-3 has previously been demonstrated to be more effective than GM-CSF in supporting colony formation of very early primitive multipotential blast cells (36). IL-3 supports several divisions of early progenitor cells, but the resulting proliferation of stem cells may rapidly regress in the absence of a second, more lineage-specific growth factor (37). IL-3, in fact, appears to act synergistically with a number of hematopoietic CSF to induce various colony formations: with IL-6 to support early blast cell colony formation, with G-CSF to support granulocytic colony formation, and with GM-CSF to support granulocyte, eosinophil, and monocyte colony formation (38, 39).

In summary, we have demonstrated that 7 d of administration of i.p. rmGM-CSF in newborn rats induces significant changes in the peripheral neutrophil count and the bone marrow NSP compared with a similar course of rmIL-3. This increase in the peripheral neutrophil count and bone marrow NSP, however, failed to be prophylactic against experimental GBS infection. Seven d of rmIL-3 failed to induce significant changes in the peripheral neutrophil count and/or the bone marrow and liver/spleen NSP. rmIL-3, however, significantly increased bone marrow CFU-GM colony formation. rmIL-3 appears to expand newborn myeloid progenitor cells, but may require the sequential addition of a later acting, lineage-specific CSF such as G-CSF or GM-CSF to optimize its induction of bone marrow myelopoiesis and inducement of peripheral neutrophilia. Optimal stimulation of neonatal hematopoiesis may require the combination of both early- and late-acting hematopoietic CSF. Enhancement of neonatal host defense may require the developmental maturation of myelopoiesis and functional activation of mature effector phagocytic circulating immune cells. Future studies are underway to develop the optimal regimen for enhancing neonatal myelopoiesis and host defense.

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