Recombinant Human Granulocyte-Macrophage Colony-Stimulating Factor Primes Neonatal Granulocytes for Enhanced Oxidative Metabolism and Chemotaxis[1]

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ABSTRACT. Recombinant human granulocyte-macrophage colony-stimulating factor (rhGM-CSF) induces proliferation and differentiation of hematopoietic stem cells. Additionally, rhGM-CSF enhances the physiologic responses of adult polymorphonuclear leukocytes (PMN) especially with respect to oxidative metabolism and chemotaxis. Neonatal PMN are deficient in chemotaxis and have been demonstrated to have reduced oxidative responses in times of stress. We evaluated the priming effects of rhGM-CSF (1–100 pmol/L) on cord (neonatal) superoxide production and chemotaxis. Cord and adult PMN were incubated with 100 pmol/L rhGM-CSF (Amgen, 4×10^7 U/mg) for 0–120 min and stimulated with N-formyl-l-methionyl-l-leucyl-phenylalanine. RhGM-CSF enhanced O_2^- production at all time periods with maximal priming at 60 min ($147.97 \pm 11.14\%$ $p \leq 0.006$) with less, but significant enhancement at 120 min ($116.53 \pm 7.92\%$ $p \leq 0.05$). Maximal adult PMN O_2^- release occurred at 120 min ($190.02 \pm 8.71\%$ $p \leq 0.003$) and was more pronounced than cord PMN. RhGM-CSF (100 pmol/L × 30 min) incubation of cord PMN also primed for increased PMN O_2^- release after zymosan-activated serum stimulation ($p \leq 0.05$) but not PMA ($p =$ NS). Co-incubating cord PMNs with 100 pmol/L rhGM-CSF and a murine anti-human GM-CSF antibody (0–100 mcg/ml) resulted in 95% inhibition of the priming effect of rhGM-CSF enhancement of cord PMN O_2^- release. RhGM-CSF, primed cord PMN for enhanced chemotaxis during early incubation (5 min) with both N-formyl-l-methionyl-l-leucyl-phenylalanine (10^{-8} M) and *Escherichia coli* filtrate ($113.54 \pm 6.11\%$ $p \leq 0.025$ and $110.84 \pm 4.69\%$ $p \leq 0.001$), respectively, but not at 30-min or 60-min incubation. These studies suggest that rhGM-CSF similarly primes neonatal PMN like adult PMN for enhanced *in vitro* PMN oxidative responses and chemotaxis. (*Pediatr Res* 26: 395–399, 1989)

Abbreviations

PMN, polymorphonuclear leukocyte (bands + neutrophils)
rhGM-CSF, recombinant human granulocyte-macrophage colony stimulating factor

O_2^-, superoxide
FMLP, N-formyl-l-methionyl-l-leucyl-phenylalanine
PMA, phorbol myristate acetate
CFU-GM, Colony forming unit-granulocyte-macrophage
SOD, superoxide dismutase
ZAS, zymosan activated serum
Ab, antibody
DMSO, dimethylsulfoxide
G-BSA, Gey's media with 2% BSA

GM-CSF is a small glycoprotein (22 000 D) which has been purified to homogeneity from a medium conditioned by the Mo T lymphoblastoid cell line (1). Recently, it has been determined that cell division and differentiation of hematopoietic progenitor stem cells are dependent on the continuous or intermittent supply of specific glycoproteins such as rhGM-CSF (2–4). Both the natural and the recombinant forms of GM-CSF stimulate granulocyte, macrophage, and eosinophil bone marrow colonies *in vitro*. (5). Although growth factors are necessary for adequate proliferation and release of bone marrow progenitor cells, they also have been demonstrated to enhance the function of mature effector cells.

Conjointly with its role of inducing proliferation and differentiation of hematopoietic stem cells, GM-CSF has been demonstrated to enhance adult host defense by improving the functional capabilities of adult mature neutrophils. Incubation of rhGM-CSF with adult PMN enhances the response of neutrophils with respect to oxidative metabolism, locomotion (chemotaxis), phagocytosis, and cytotoxicity (6, 7). Specifically, GM-CSF augments FMLP, C5a, and leukotriene B₄-induced superoxide anion production (8, 9), enhances FMLP stimulated chemotaxis (9), and promotes phagocytosis of opsonized *Staphylococcus aureus* (10). GM-CSF has also recently been demonstrated to induce the expression of surface active adhesion glycoproteins such as C3bi resulting in the acceleration of adult PMN aggregation (11). Unfortunately, all of the above enhanced PMN activity primed by rhGM-CSF, have been demonstrated with adult PMN and little is known about the potential effect of rhGM-CSF with neonatal PMNs.

Neonatal host defense in the animal and human neonate is immunologically immature and contributes significantly to the high incidence of overwhelming sepsis in the preterm and term newborn (12). Humoral and phagocytic immunity are both deficient in neonatal host defense. A recent review by Hill (13) summarized the variety of biochemical, structural, and functional abnormalities that have been described in neonatal PMN.

Inasmuch as rhGM-CSF has been demonstrated to prime adult PMN for increased functional activity, we examined the effects of rhGM-CSF on the modulation of PMN physiologic activity in the newborn. This study investigated the biologic effects of rhGM-CSF on cord (neonatal) PMN oxidative metabolism, chemotaxis, and bacterial killing.

MATERIALS AND METHODS

RhGM-CSF. rhGM-CSF supplied by Amgen Biologicals, Thousand Oaks, CA, is expressed in *Escherichia coli* and purified to apparent homogeneity before formulation in 0.025% human serum albumin and PBS. Purity of rhGM-CSF was confirmed to be more than 95% by the presence of a single protein band by SDS-PAGE. Biologic activity of 4×10^7 U/mg was determined by CFU-GM of nonadherent human bone marrow cells or by KG-1 colony formation in semisolid media with activity at 10^{-11} to 10^{-12} M. GM-CSF was shown to be free of measurable endotoxin by the Limulus amebocyte lysate assay. GM-CSF was used at concentrations in the range of 1-100 pm/L reflecting the reported enhancement of adult neutrophil oxidative metabolism in response to FMLP to study its effect on cord and adult neutrophil activity (14).

Materials. PMA, FMLP, and zymosan particles were purchased from Sigma Chemical Co. (St. Louis, MO). PMA was dissolved in DMSO, 1 mg/mL, aliquoted, and stored at $-20°C$ until used at a final concentration of 2 μM. FMLP was dissolved in DMSO at 10^{-2} M, aliquoted and stored at $-20°C$, and finally used at final concentrations of 10^{-6} to 10^{-9} M. ZAS was prepared by boiling zymosan particles suspended in normal saline for 1 h. The particles were cooled, centrifuged and resuspended in normal saline for storage at $-20°C$. Autologous serum was later added to the zymosan particles, and incubated for 30 min at 37°C. The particles were then centrifuged and the serum removed for assay, at a final concentration of 10%. *E. coli* filtrate was prepared by culturing the microorganism to maximum phase growth (24 h) in brain heart infusion (Difco, Detroit, MI), centrifuging the culture, and collecting the supernatant broth which was then filtered sterilely, aliquoted, and stored at $-20°C$ (final concentration 10% solution).

Anti-GM-CSF MAb. Anti-GM-CSF MAb (Genzyme, Boston, MA) is a murine MAb generated against purified rhGM-CSF, and is of the IgG1 subclass. It was received as 95% pure as determined by SDS gel electrophoresis, and is neutralizing for GM-CSF activity (hematopoiesis). Complete neutralization of 50 U of GM-CSF could be obtained with 1 μg of antibody, and no detectable cross-reaction with human G-CSF, IL-3, or mouse GM-CSF can be measured. The antibody was diluted to concentrations of 0.32 to 100 μg/mL to study its inhibitory effect on the priming of GM-CSF in neutrophil superoxide production.

Isolation of human PMN. Heparinized venous blood was drawn from normal volunteers in accordance with the principles of the Declaration of Helsinki. Venous cord blood was drawn from the umbilical vessels of placentas of normal, full-term, nonstressed infants immediately after vaginal delivery or cesarean section. Both cord and adult donor red blood cells were allowed to sediment, after the addition of 6% Dextran-70 in 0.9% saline (McGaw Laboratories, Irvine, CA). The leukocyte-enriched plasma was collected, layered on a Ficoll-Hypaque gradient ($p = 1.077$), and centrifuged for 15 min. PMN were recovered in the cell pellet, and any remaining erythrocytes were hemolyzed by hypotonic lysis. Isolated PMN were then suspended in Dulbecco's pH 7.4, or PBS, pH 7.4 with 5 mM glucose at various concentrations depending on particular assay requirements. PMN isolated by density gradient separation were purified to greater than 98% homogeniety. Cell viability as measured by trypan blue exclusion was more than 99%. PMN were used in the following assays within 2 h of collection.

Superoxide production. Superoxide release was assessed as the SOD sensitive reduction of ferricytochrome C as previously described (15, 16). Aliquots of PMN were exposed to various concentrations of GM-CSF for 5-120 min at 37°C. Cytochrome C (1.19 mM) and cytochalasin B (5 μg/mL) were added, and the cells were stimulated by either PMA, FMLP, or ZAS at concentrations given above, at 37°C for 5 min. To arrive at a nonreactive control value, one sample of each treatment received 100 μg of SOD. The reaction was stopped with the addition of N-ethylmalemide (1 mM) (Sigma), and absorption at 550 nm of the sample supernates was measured on a Gilford spectrophotometer (Oberlin, OH). Results are expressed as nmol reduced cytochrome C using the extinction coefficient of 21.1×10^3 M^{-1} cm^{-1}. Control values of PMN incubated with PBS and 0.025% human albumin and cytochalasian B were obtained at each time point studied in all assays of superoxide production.

To determine if the observed effects of rhGM-CSF were primarily due to the CSF rather than extraneous stimulation from contamination by other stimulants, graded concentrations of anti-GM-CSF antibody and PBS control were preincubated vol/vol with and without GM-CSF for 1 h at room temperature. Cord neutrophils were then exposed to the antibody/GM-CSF mixtures for 30 min at 37°C, and superoxide release was measured as described above.

Granulocyte chemotaxis. The Gallin modification of the Boyden chamber technique was performed to assay for chemotaxis activity (17). The Boyden apparatus was prepared by inserting two filters (0.4 and 5.0 μm, Nucleopore, Pleasanton, CA) to separate the upper and lower chambers. Adult or cord PMN were labeled with sodium 51-chromate (ICN Radiochemicals, Irvine, CA), and suspended in G-(GIBCO, Grand Island, NY) BSA (Difco, Detroit, MI). Appropriate amounts of GM-CSF or 0.025% albumin in normal saline were then added, and the cells were incubated for 5 to 120 min at 37°C. The lower chamber of the Boyden apparatus was then filled with a chemoattractant solution containing G-BSA, 1.0% donor or cord serum, and either 10% *E. coli* filtrate or FMLP (10^{-6} to 10^{-9} M). The treated cells were added to the upper chamber. After incubation at 37°C for 90 min, the filters were separated and the 0.4-μm filters containing the trapped migrating cells were counted in a Beckman LS1800 scintillation counter (Beckman Instruments Inc., Fullerton, CA). Aliquots of 100% chromium labeled PMN were analyzed in the same manner, and results are expressed as percent chemotaxis, *i.e.* migrated cell cpm/100% labeled cell cpm.

Bactericidal activity. Bacterial killing capability was measured by a modification of the Quie method, as described previously (18). Briefly, *Staphylococcus aureus* (American Type Culture no. 27217, Rockville, MD) was subcultured to obtain maximum phase growth, diluted in Kreb's ringers phosphate buffer with 0.1% glucose and 1.0% BSA at a concentration of approximately 2×10^8 bacteria/mL, OD 590. Bacteria were opsonized with control or cord serum for 10 min before the assay. Aliquots of PMN were isolated from donor or cord blood before the Ficoll separation, and incubated with various doses of GM-CSF for 5-120 min at 37°C. The treated and untreated cells were then mixed with the opsonized bacteria at a ratio of 1:4 and incubated for 15 min at 37°C. Aliquot samples of bacteria and cell suspension were serially diluted in sterile water, and finally plated in warm nutrient agar (Difco, Detroit, MI). Colony growth was measured after overnight incubation, and results are expressed as percent bacteria killed.

Statistical analysis. All results are expressed as mean values ± SEM for three or more experiments. The probability of significant differences when examining treatment groups was determined with the use of the unpaired Student's *t* test; whereas 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 test to define the unique subsets within the study. Statistical analysis was performed using the Biostat-I statistical program (Sigma Soft, Placentia, CA) for the IBM personal computer. *p* values ≤0.05 were considered significant.

RESULTS

Effect of rh-GM-CSF on superoxide production in neonatal PMN. To determine the effect of incubating cord PMN with various concentrations of GM-CSF, a dose response of rhGM-CSF (5, 10, 50, 100 pmol/L), incubated with neonatal PMN followed by stimulation with FMLP (10^{-7} M) was undertaken. A significant effect occurred at 10 pmol/L [152.54% control (10 pmol/L)] and this enhanced effect continued up to 100 pmol/L [130.34 ± 7.40% control (100 pmol/L)]; $p \le 0.001$ and ≤ 0.001, respectively. We next used 100 pmol/L of rhGM-CSF incubated with cord PMN and then stimulated with FMLP (10^{-7} M) to study the time response of GM-CSF incubation. These studies demonstrated maximal priming to occur between 30 and 60 min ($p \le 0.006$) with less, but still significant enhancement at 120 min ($p \le 0.05$) (Fig. 1). After selecting a maximal time of incubation (30 min), we studied the dose response of FMLP ($10^{-9}-10^{-6}$ M) of cord PMN incubated with 100 pmol/L rhGM-CSF. These experiments demonstrated significant enhancement at both 10^{-7} and 10^{-6} M, ($p \le 0.005$) and ($p \le 0.001$), respectively.

Next, we varied the agonist to determine if 100 pmol/L rhGM-CSF incubation for 30 min with neonatal PMN resulted in significant enhancement with other stimulants. ZAS but not PMA stimulation resulted in significant enhancement of rhGM-CSF treated neonatal PMN superoxide generation (Table 1). In an attempt to abrogate the effects of priming neonatal PMN with rhGM-CSF, and to eliminate any other contaminating cause for PMN functional activation, we coincubated neonatal PMN with 100 pmol/L rhGM-CSF and studied the dose response of adding additional anti-rhGM-CSF Ab (2, 32, 64, 100 μg/mL) followed by FMLP stimulation (10^{-7} M). Anti-rhGM-CSF antibody itself had no effect on neonatal PMN superoxide generation (149.2 ± 11 *versus* 138.9 ± 14 nmol/10^7 cells) (100 μg/mL Ab *versus* control) p = NS. However, anti-GM-CSF antibody coincubated with rhGM-CSF resulted in significant inhibition of enhanced superoxide anion production at 32, 64, and 100 μg/mL Ab resulting in $\le 95\%$ inhibition at 100 μg/mL (Fig. 2; Table 1).

Incubating rhGM-CSF 100 pmol/L for 0–120 min with adult PMN demonstrated significant enhancement of adult PMN superoxide generation at 30, 60, and 120 min. In contrast, however, similar doses of rhGM-CSF resulted in an earlier but less maximal response with neonatal PMN (30–60 min) compared to the priming effect of rhGM-CSF with adult PMN (Fig. 1).

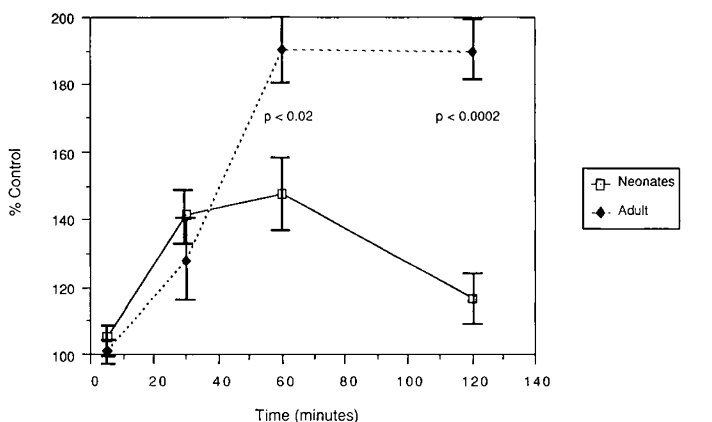*Effect of rhGM-CSF on chemotaxis in neonatal PMN.* The

Fig. 1. Comparison of adult *versus* cord neutrophil superoxide production after exposure to rhGM-CSF. Adult and cord neutrophils (1 × 10^7/mL) were incubated for 30 min with 100 pmol/L rhGM-CSF 37°C. After the addition of cytochrome C (1.19 mM) and cytochalasin B (5 μg/mL), the cells were stimulated with FMLP (10^{-7} M) for 5 min. Results are expressed as mean percent of control ± SEM of five experiments done in triplicate. Cord control 138.86 ± 13.9 nmol/10^7 cells at 30 min. Adult control 124.72 ± 14.4 nmol/10^7 cells at 120 mm.

Table 1. *Neutrophil superoxide production (mean ± SEM) with different agonists after stimulation with and without GM-CSF and antibody (p < 0.05 is considered significant)**

		O_2^- Production (nmol/10^7c)	p value
FMLP	Control	199.6 ± 17.7	0.001† (n = 5)
	GM-CSF	285.3 ± 35.8	
ZAS	Control	174.94 ± 14.1	<0.05† (n = 5)
	GM-CSF	182.39 ± 14.3	
PMA	Control	307.20 ± 24.0	NS (n = 3)
	GM-CSF	335.28 ± 14.8	
FMLP	Control + Ab	149.16 ± 11.2	NS (n = 6)
	GM-CSF + Ab	144.93 ± 12.0	

* Superoxide production of cord PMN after incubation with 100 pM/L rhGM-CSF or anti-GM-CSF/GM-CSF and stimulation with various agonists. 1 × 10^7/mL PMN were incubated for 30 min with rhGM-CSF at 37°C. Cytochrome C (1.19 mM) and cytochalasim B (5 μg/mL) were added, and the cells stimulated with FMLP (10^{-7} M) for 5 min or, ZAS (10% solution) or PMA (2 μM) for 10 min. To abrogate the effect of rhGM-CSF, 100 μg/mL of anti-GM-CSF antibody was incubated with the growth factor for 1 h at room temperature and the antibody rhGM-CSF mixture was added to cord PMN for another 30-min incubation. The cells were then stimulated with FMLP (10^{-7} M) for 5 min as described above. Results are expressed as nmol reduced cytochrome C/10^7 cells, and are presented as mean values ± SEM of four to six experiments done in triplicate.

† p values were calculated from data expressing superoxide produced as percentage of control.
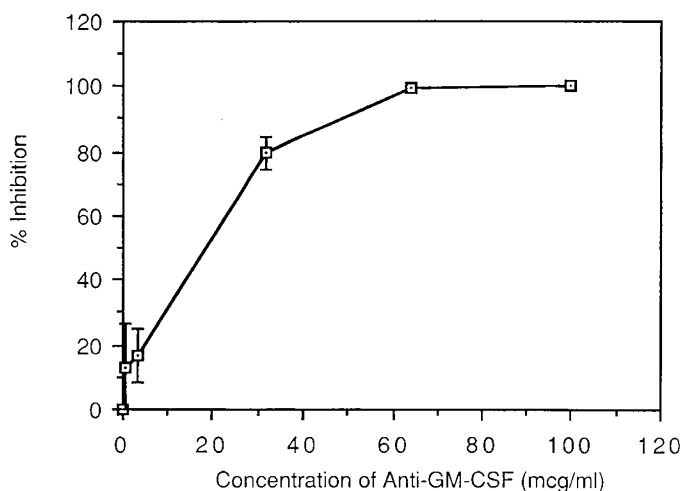
Fig. 2. Effect of graded concentrations of anti-rhGM-CSF antibody on the modulation of GM-CSF action on superoxide production of cord neutrophils. Concentrations of anti-GM-CSF antibody 0–100 μg/mL were preincubated with the growth factor (vol/vol) for 1 h at room temperature. Cord neutrophils were then exposed to the antibody/GM-CSF mixture for 30 min at 37°C. Superoxide release was measured as described in Figure 1. Results are expressed as mean percent of control ± SEM of six experiments done in triplicate.

time course of rhGM-CSF enhancement of neonatal PMN chemotaxis is shown in Figure 3. Significant priming by rhGM-CSF (100 pmol/L) with neonatal PMN and FMLP as the chemoattractant occurred very early (5 min) ($p \le 0.025$) with a return to normal activity after 30- or 60-min incubation (Fig. 3). A second agonist, *E. coli* filtrate, also resulted in significant rhGM-CSF enhancement of neonatal PMN chemotaxis after 5-min incubation (110.84 ± 4.69% of control) ($p \le 0.05$) with a return

to normal at 30- and 60-min incubation. This rapid enhancing effect of rhGM-CSF on neonatal PMN locomotion (chemotaxis) was in contrast to a later and more pronounced effect seen with rhGM-CSF and neonatal PMN superoxide release.

Effect of rhGM-CSF on bacterial killing of neonatal PMN. Neonatal PMN incubated with unopsonized *Staphylococcus aureus* were unable to efficiently kill bacteria with or without rhGM-CSF (data not shown). Neonatal PMN were then challenged with opsonized bacteria (autologous serum) following 100 pmol/L rhGM-CSF incubation for 0–120 min. Enhancement of neonatal PMN bacterial killing was observed after 120-min incubation but not at 30 or 60 min of incubation with rhGM-CSF; at 120 min, $n = 3$ 120.9 ± 5.4% of control ($p \le 0.05$) (control *versus* rhGM-CSF) (Fig. 4).

DISCUSSION

Numerous *in vitro* abnormalities have been demonstrated in neonatal PMN, especially in times of stress or infection. These deficits include decreased deformability and chemotaxis (19, 20), defective opsonic activity and phagocytosis (21), a discrepancy between superoxide and hydroxyl radical generation (22), reduced oxidative responses in stressed newborns (23) and impaired bacterial killing in newborns stressed or concurrently infected (24). These qualitative and additional quantitative abnormalities in neonatal myeloid bone marrow precursors and circulating PMN have suggested the need for additional adjuvant therapy in the treatment of overwhelming neonatal sepsis (25).

RhGM-CSF administered as a daily intravenous infusion, has recently been shown to enhance peripheral PMN oxidative metabolism in primates (26) and correct defects in phagocytosis and bacterial killing in patients with AIDS *in vivo* (27). We postulated that rhGM-CSF might also prime neonatal PMN for enhanced functional activity. RhGM-CSF (1–100 pmol/L), by itself, was not found to be a direct stimulant of neonatal PMN. When rhGM-CSF was incubated with cord PMN before being stimulated by a chemoattractant, rhGM-CSF primed neonatal PMN for enhanced superoxide production with FMLP, ZAS, but not PMA stimulation. The results in this neonatal study were similar to those effects seen in adult PMNs primed with rhGM-CSF (8). The difference between rhGM-CSF priming neonatal and adult PMN is the degree and time of maximal enhancement. However, any differences between our cord PMN studies and adult PMN studies should be taken with caution because cord PMN may be
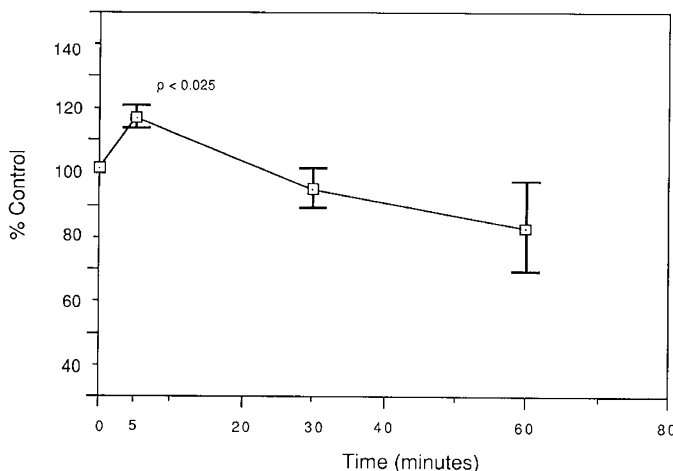

Fig. 3. Effect of rhGM-CSF on chemotaxis in neonatal neutrophils. The Gallin modification of the Boyden chamber technique was used. Cord PMN (1 × 10^7/mL) were labeled with sodium 51-chromate and incubated for various time intervals (0–60 min) with 100 pmol/L rhGM-CSF. Cells were placed in the Boyden chamber with FMLP (10^{-8} M) as the chemoattractant and incubated for 90 min. Results are expressed as mean percent of control ± SEM of five experiments done in triplicate.
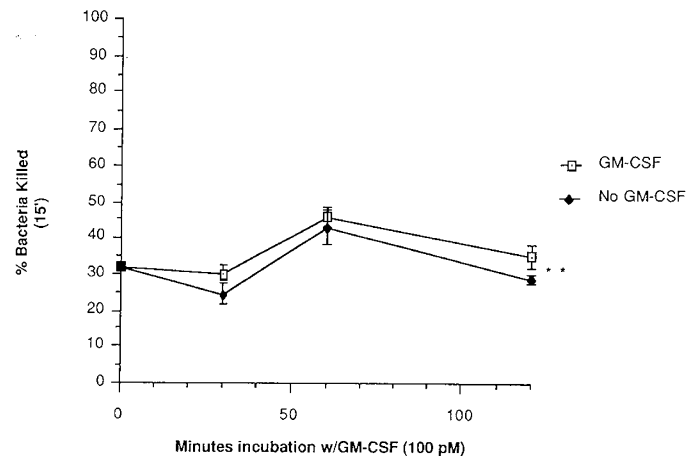

Fig. 4. Bacterial killing capability was measured by a modification of the Quie method as described. *Staphlococcus aureus* was subcultured and opsonized with cord or control serum. PMN were incubated at various times with 100 pM GM-CSF at 37°C. The treated and untreated cells were mixed with the opsonized bacteria and killing was allowed to progress for 15 min. Colony growth was measured after overnight incubation and results are expressed as mean percent bacteria killed ± SEM for six experiments.

in a more preactivated state compared to newborn peripheral PMN or adult peripheral PMN. Our adult PMN studies like Weisbart *et al.* (8), demonstrated that maximal enhancement occurred at a later time interval of incubation, 60–120 min, and was more pronounced compared to neonatal PMN. This difference in maximal enhancement in adults *versus* neonatal PMN may be secondary to increased amounts of superoxide generation in newborn PMN *versus* that found in adult PMN (22, 28).

Our studies corroborate the adult PMN results of Weisbart *et al.* (8, 9) who reported that GM-CSF primed for increased superoxide generation after 4–5 min after FMLP stimulation. Although our results only represent the effect of GM-CSF priming superoxide generation in cord PMN after 5 min of FMLP stimulation, previous adult PMN data have also shown this priming effect to occur in a continuous assay. The failure of rhGM-CSF to prime neonatal PMN for enhanced effects with PMN stimulation may in part be secondary to a normally increased response of PMA-stimulated PMN superoxide release compared to other agonists (Table 1). Weisbart *et al.* (9) also failed to document enhancement of rhGM-CSF with PMA stimulation in adult PMN. He noted that the rhGM-CSF enhanced effect might be related to increased expression of surface active chemotactic receptors (FMLP) (9). This hypothesis is consistent with the fact that PMA has a direct effect on protein kinase C activation, by passing the need for upregulation and expression of surface active receptors necessary for signal transduction commonly demonstrated during FMLP and ZAS stimulation.

Neonatal PMN chemotaxis in this study seem to parallel the effects found with rhGM-CSF enhancement with adult PMN (9). Incubation with 100 pmol/L rhGM-CSF enhanced early (within 5 min) chemotaxis with both FMLP and *E. coli* filtrate as chemoattractants in neonatal PMN compared to maximal enhancement at 5–15 min with 100 pmol/L rhGM-CSF with adult PMN. Weisbart *et al.* (9) demonstrated a 3-fold increase in the expression of high affinity FMLP surface receptors during a 5- to 15-min exposure of 100 pmol/L rhGM-CSF with adult PMN. Additionally, Weisbart *et al.* (9) studied the effects of more prolonged incubations of rhGM-CSF (1–2 h) with adult PMN and concluded that the increase in oxidative metabolism seen at 1–2 h post-rhGM-CSF incubation was secondary to an increase of low affinity FMLP surface receptors.

Bacterial killing was enhanced after a 120-min incubation of neonatal PMN with 100 pmol/L rhGM-CSF. Fleischmann *et al.*

(10) reported enhanced phagocytosis of opsonized *S. aureus* with 100 pmol/L rhGM-CSF after 15 and 120 min incubation with adult PMN. However, they did not document any increase in intracellular killing at 30, 60, or 120 min. Baldwin *et al.* (27), however, administered daily intravenous rhGM-CSF to AIDS patients with defects of PMN bacterial killing and documented a correction of their *in vitro* abnormality. It is intriguing that our study demonstrated that rhGM-CSF increased neonatal PMN chemotaxis, superoxide production, and intracellular bacterial killing in a temporal fashion resulting in an early effect on PMN locomotion, then a later maximal effect on increased PMN oxygen radical formation eventually resulting in a much later effect on bacterial killing.

The mechanism by which rhGM-CSF primes PMN for enhanced physiologic responses is still under investigation. Naccache *et al.* (29) recently demonstrated that rhGM-CSF inhibits the cytoplasmic alkalinization which commonly follows stimulation with agonists such as FMLP, PMA, LTB₄, and also potentiates the mobilization of intracellular calcium. Sullivan *et al.* (30) reported that rhGM-CSF primes adult PMN by increasing the rate of membrane depolarization induced by FMLP and enhances release of arachidonic acid from PMN plasma membranes. These latter findings are consistent with Dahinden *et al.* (31) who recently demonstrated an increase in leukotriene production in human adult PMN primed with rhGM-CSF. Additionally, rhGM-CSF does not directly stimulate protein kinase C activity (30). Although the mechanism of rhGM-CSF priming PMN is unknown, it is speculative to suggest that following upregulation and expression of surface active receptors (9), rhGM-CSF enhances the increase in a second messenger such as an arachidonic acid metabolite or intracellular calcium. This increase in a second messenger may activate signal transduction pathways in human PMN and subsequently enhance physiologic activity.

This study has demonstrated that GM-CSF moderately enhances neonatal oxidative metabolism, and mildly improves chemotaxis and bacterial killing. RhGM-CSF is currently undergoing *in vivo* evaluation in adults after intensive chemotherapy regimens, bone marrow transplantation, and additionally to treat patients with AIDS, aplastic anemia, and myelodysplastic syndromes. However, few *in vivo* trials with GM-CSF have been studied in children. Our preliminary results, however, suggest that rhGM-CSF does enhance *in vitro* neonatal PMN functional activity and warrants further *in vitro* neonatal myeloid investigations.

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