The Effects of Recombinant Human Granulocyte-Macrophage Colony Stimulating Factor on *in Vitro* Cord Blood Granulocyte Function

ROBERT W. FRENCK, JR., E. STEPHEN BUESCHER, AND SAROJ VADHAN-RAJ

Program in Infectious Diseases and Clinical Microbiology, University of Texas Medical School at Houston [R.W.F., E.S.B.] and Department of Clinical Immunology and Biological Therapy, M.D. Anderson Hospital, Houston, Texas [S.V.-R.]

ABSTRACT. Neonatal granulocytes are recognized to have functional defects which are thought to be important in the increased susceptibility to infection in the neonate. Recombinant human granulocyte-macrophage colony-stimulating factor (rhGM-CSF), a member of a family of glycoproteins essential for the *in vitro* survival, proliferation, and differentiation of hematopoietic progenitor cells, has been shown to enhance the functional capabilities of adult granulocytes. This study tested the effects of rhGM-CSF on the locomotion, superoxide generation, phagocytosis, bactericidal activity, and membrane depolarization responses of cord blood granulocytes. Concentrations of rhGM-CSF between 100 and 1 pM significantly enhanced the leading front of cord blood granulocyte locomotion. The mean distance migrated by the cell population and the number of cells responding to the chemoattractant were also significantly enhanced in cord blood granulocytes treated with 1 pM rhGM-CSF. Superoxide anion production was significantly enhanced in cord blood granulocytes stimulated with fMLP after a 30- or 60-min exposure to 100 pM rhGM-CSF. However, this enhancing effect was not observed in cells incubated with rhGM-CSF for 2 h before formyl-methionine-leucine-phenylalanine stimulation. Phagocytosis, bactericidal activity, and membrane depolarization responses of cord blood granulocytes were not affected by exposure to rhGM-CSF. These findings demonstrate that selected cord blood granulocyte functions are enhanced by *in vitro* exposure to rhGM-CSF. Whether these *in vitro* observations have *in vivo* significance await further study. (*Pediatr Res* 26: 43-48, 1989)

Abbreviations

rhGM-CSF, recombinant human granulocyte-macrophage colony stimulating factor
HBSS, Hank's Balanced Salt Solution
fMLP, Formyl-methionine-leucine-phenylalanine
AcS, zymosan-activated serum
A+f, zymosan-activated serum plus fMLP

CSF are glycoproteins essential for the *in vitro* survival, proliferation and differentiation of hematopoietic progenitor cells (1, 2). One of these, GM-CSF has multilineage proliferation effects

in vitro, stimulating granulocyte, macrophage, eosinophil, megakaryocyte, and erythroid colony growth.

Recently the gene for hGM-CSF has been cloned (1, 3) allowing biosynthetic production of a recombinant product. For the first time, large quantities of pure GM-CSF are available, making it possible to more thoroughly study and characterize this glycoprotein. As a result, it is now appreciated that in addition to its proliferative effects, GM-CSF has functional effects on mature granulocytes. It increases phagocytosis (4, 5), enhances fMLP-stimulated superoxide production (6), causes lysozyme secretion (4), alters membrane depolarization responses (7), alters adhesion (8), and is itself a weak granulocyte chemoattractant (10). GM-CSF has also been identified as the neutrophil migration inhibition factor from T cells (NIF-T) (9), a lymphokine that suppresses granulocyte migration.

Despite evaluation of the effects of GM-CSF on granulocytes obtained from adults, little information exists on the effects of this protein on neonatal granulocytes. Since neonatal granulocytes are widely recognized to be functionally less competent than adult cells (11-19) and because GM-CSF enhances certain adult granulocyte functions, we hypothesized that GM-CSF might also enhance neonatal granulocyte functions. We therefore performed the following studies to examine the effects of *in vitro* GM-CSF exposure on umbilical cord blood granulocyte function.

MATERIALS AND METHODS

Isolation of granulocytes. Heparinized (1 U/ml) umbilical cord blood was collected after birth by venipuncture of the umbilical cord of healthy, term infants. Paired samples of heparinized blood were obtained from healthy adult volunteers as controls. Granulocytes were purified by Hypaque (Winthrop Breon Lab, NY)-Ficoll (Sigma Chemical Co., St. Louis, MO) gradient centrifugation (20), followed by 3% dextran (Sigma) sedimentation and hypotonic lysis. The resulting cells were washed and resuspended in HBSS without Ca^{++} or Mg^{++} (HBSS without).

rhGM-CSF. rhGM-CSF was prepared and provided by Immunex Corp (Seattle, WA). hGM-CSF cDNA was isolated from a library constructed of mRNA from the human T cell line HUT-102 inserted into and expressed in yeast (21). The purified rhGM-CSF has a specific activity of approximately 5×10^7 colony-forming U/mg of protein (22). Its mol wt was assumed to be 22 kD. A stock GM-CSF solution was prepared by dissolving the lyophilized GM-CSF in normal saline at a concentration of 4.54 M. Aliquots of stock were kept frozen at -70°C until used. Endotoxin contamination was <1 ng/mg of lyophilized GM-CSF as measured by the Limulus amoebocyte lysate assay.

Granulocyte locomotion. Locomotion was measured using a

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Correspondence Robert W. Frenck, Jr., M.D., Pediatric Infectious Diseases, University of Texas Medical School at Houston, 6431 Fannin Street, Room 1.739, Houston, TX 77030.

modified under-agarose assay (23). One part Eagle's minimal essential medium ($2 \times$ concentration) was combined with one part agarose (0.24 g/10 ml H_2O) containing 10% autologous serum. rhGM-CSF in appropriate concentrations was added to the agarose mixture (creating a uniform concentration of rhGM-CSF throughout the agarose) and 5-ml aliquots of agarose were placed into quadruplicate 60×15 mm Petri dishes. Four pairs of parallel troughs were cut into each agarose plate, and 20 μ l of granulocytes (suspended in MEM and 10% autologous serum at 2.5×10^7 /ml) were placed in the inner trough, the locomotive stimulus was placed in the outer. The locomotive stimuli used were HBSS without, AcS, 5×10^{-7} M fMLP (Sigma) and activated serum plus fMLP (A+f). To evaluate the chemoattractant properties of rhGM-CSF, the locomotive stimuli AcS, fMLP, and A+f were replaced by 10 nM, 100 pM, and 1 pM rhGM-CSF in the outer trough of the agarose plate. The rhGM-CSF diffused into the agarose presenting the cell with a gradient of the glycoprotein. After 4 h of incubation at 37°C, the plates were fixed for 5 min with formalin and the cells were stained with Diff-Quik (Scientific Products, McGaw Park, IL). Leading front distances were measured from projected images of the stained plates and quadruplicate measurements were averaged.

Image analysis of locomotion. Triplicate fixed, stained chemotaxis plates were scanned on a computerized, automated image analysis system that utilizes a high resolution video camera (6 μ /pixel) (24). The video camera scans an area 400 pixels wide by 400 pixels deep, starting at the edge of the cell trough and progressing toward the stimulus trough. The computer assigns a value from 1 to 256 (1 = white, 256 = black) to each pixel which corresponds to the cell density in that pixel. The density information in each 400 pixel wide swath is then averaged and plotted as a function of distance from the cell trough. Simultaneously, the leading front distance, the mean distance migrated by the cells, and the area under the curve (density $\times$ distance migrated) are measured and calculated.

Granulocyte superoxide production. Purified granulocytes (2.5×10^6 cells/ml) were suspended in HBSS without, which contained 10% heat inactivated autologous serum and incubated at 37°C with either diluent or GM-CSF. At times 0, 30, 60, 90, and 120 min, cord blood and adult granulocytes were aliquoted into three pairs of tubes (1×10^6 cells/tube). Superoxide dismutase (Sigma) (0.025 mg/ml final concentrations) was added to one tube of each triplicate pair. fMLP was added to all of the tubes, followed by 0.1 ml cytochrome c (12.5 mg/ml). The triplicate pairs of tubes were tumbled for 15 minutes at 37°C and then the cells were pelleted ($18,000 \times g$, 30 s) and discarded. The supernate was examined for reduced cytochrome c at 550 nm using a Gilford 25 spectrophotometer. The triplicate determinations were averaged and superoxide anion production per million cells per 15 min was then calculated using an extinction coefficient of 21 (25).

Granulocyte membrane depolarization assay. Membrane depolarization responses were measured using the carbocyanine dye DiOC₅ (3) (26). Purified granulocytes were suspended in HBSS without and incubated with rhGM-CSF or diluent at 37°C. At times 0, 30, 60, and 120 min, aliquots of cells (2.5×10^5 cells/ml final concentration) were added to HBSS with Ca⁺⁺ and Mg⁺⁺ containing 2.5×10^{-7} M DiOC₅ (3) (Molecular Probes, Eugene, OR) for 5 min at 37°C. After transfer to a cuvette, 2 ml of cells were placed in a Perkin-Elmer fluorometer (Perkin-Elmer Corp., Norwalk, CT) with continuous stirring. After stabilization of the baseline, 20 μ l of fMLP stock were added and the reaction was allowed to continue for 5 min with continuous recording of fluorescence. Membrane depolarization responses are expressed as the percent decrease in fluorescence, calculated as Δ fluorescence/starting fluorescence.

Granulocyte phagocytosis assay. Purified granulocytes (1×10^6) were suspended in HBSS with Ca⁺⁺ and Mg⁺⁺ containing 10% autologous serum and were incubated for 30 min at 37°C

with either diluent or GM-CSF. Boiled *Staphylococcus aureus* (1×10^8) were then added and the mixture was tumbled at 37°C for 30 min. Aliquots of the mixture were removed at 5-min intervals, and each was combined with 20 U of lysostaphin (ICN, Cleveland, OH) to remove extracellular bacteria (27). This mixture was incubated at 37°C for 30 min then placed on ice until cytocentrifuge slides were prepared. After staining (Diff-Quick), 200 granulocytes were counted, scoring each for the presence or absence of intracellular bacteria.

Granulocyte bacterial assay. Triplicate samples of purified granulocytes (5×10^6 final conc.) were suspended in 1 ml of HBSS with Ca⁺⁺ and Mg⁺⁺ containing 10% autologous serum and either diluent or GM-CSF. Live *S. aureus* (1×10^7 organisms) were added and the mixture tumbled at 37°C. Control mixtures, to determine 100% bacterial survival, were incubated without granulocytes and were plated at 10 minutes. Aliquots of the test mixtures were removed at 20, 45, and 90 min and lysed in sterile water. Samples of lysate were placed in 60×15 mm Petri dishes and overlaid with warm trypticase-soy agar. Plates were incubated overnight at 37°C, after which colonies of *S. aureus* were counted and bacterial survival was expressed as a percent of the control plate colony counts.

Statistical evaluation. Unless otherwise stated, all data shown are the mean $\pm$ SEM of (n) determinations and data are compared using either a two-tailed Student's *t*-test or a paired sample *t*-test (when natural pairing occurs) with $p < 0.05$ taken as a significant difference.

RESULTS

Cell purification. Samples of cord blood yielded 5.75 ± 0.51 million purified cells per milliliter of blood ($n = 50$). Purified granulocyte fractions from cord blood contained 88 ± 1.6 percent granulocytes ($n = 12$). Adult control granulocyte preparations were typically $>90\%$ pure.

Effects of GM-CSF on granulocyte locomotion. *In vitro* rhGM-CSF exposure affected cord blood granulocyte locomotion in a dose-dependent fashion (Fig. 1). Significant suppression of chemotaxis toward AcS, fMLP, and A+f resulted from exposure to 10 nM GM-CSF ($p < 0.05$ AcS, $p < 0.01$ fMLP and A+f, paired sample *t*-test and Wilcoxon signed rank test) as compared to the same cells unexposed to rhGM-CSF. Cord blood granulocytes exposed to 1 pM GM-CSF showed enhanced leading front distances compared to cord granulocytes not exposed to GM-CSF: AcS: $115 \pm 6\%$ of unexposed ($p < 0.025$), fMLP: $119 \pm 7\%$ ($p < 0.01$), and AcS + fMLP: $121 \pm 8\%$ ($p < 0.01$) (paired sample *t*-test and Wilcoxon signed rank test). Unstimulated locomotion (Fig. 1A) was significantly enhanced when cord blood granulocytes were exposed to 100 pM rhGM-CSF ($p < 0.02$, paired sample *t*-test and Wilcoxon signed rank test).

Three under-agarose chemotaxis experiments were performed testing rhGM-CSF as a chemoattractant. The total concentration of 100 pM rhGM-CSF acted as a weak but significant chemoattractant when compared to cells undergoing unstimulated locomotion toward HBSS ($108 \pm 2\%$ 100 pM rhGM-CSF stimulated cells/HBSS (unstimulated) cells, $p < 0.05$). Chemotactic responsiveness to 10 nM and 1 pM rhGM-CSF was equivalent to unstimulated locomotion (106 ± 4 and $102 \pm 1\%$, respectively).

Three experiments were performed examining cord cell chemotaxis by image analysis. Cells exposed to 1 pM rhGM-CSF then stimulated by fMLP increased their mean distance migrated and the area under the curve (number of granulocytes responding) to $155 \pm 7\%$ ($p < 0.05$) and $220 \pm 65\%$ ($p < 0.05$) of unexposed cell values, respectively. Granulocytes exposed to 1 pM rhGM-CSF then stimulated by activated serum or activated serum plus fMLP had an increased mean distance migrated by the population of cells and number of granulocytes responding

Fig. 1. The effects of rhGM-CSF on cord blood granulocyte locomotion. In each panel the locomotive stimulus is identified in the upper left corner. The vertical axis represents movement of granulocytes exposed to rhGM-CSF divided by the movement of the same population of granulocytes that had not been exposed to rhGM-CSF. Values of more than 100 reflect enhanced chemotaxis by rhGM-CSF treated cells whereas values less than 100 reflect suppression. At each concentration, squares represent the results of individual cord blood samples, each sample only being used once at a given concentration of rhGM-CSF. Bars show the mean locomotion of the individual experiments at each concentration of rhGM-CSF tested. Asterisk denotes $p < 0.05$ as determined by paired sample t -test.

to the chemoattractant but the results were not statistically different from controls.

Figure 2 depicts the results of a representative image analysis experiment. The unshaded area represents the enhanced number of granulocytes responding to each chemoattractant after incubation with 1 pM rhGM-CSF.

Despite the enhancement of locomotive responses, exposure of cord blood granulocytes to 1 pM rhGM-CSF did not normalize their locomotive responses to those observed in adult granulocytes (Table 1).

Effects of rhGM-CSF on granulocyte superoxide production. rhGM-CSF was not a stimulus for superoxide production by either adult or cord blood granulocytes (0.0 vs 0.2 nmol superoxide, adult granulocytes $\pm$ 100 pM rhGM-CSF exposure ($n = 4$) and 0.1 vs 0.5 nmol superoxide, cord blood granulocytes $\pm$ 100 pM rhGM-CSF ($n = 5$). Incubation of cord blood granulocytes with 100 pM rhGM-CSF for 30 and 60 min before fMLP stimulation significantly enhanced superoxide production by these cells (30 min, $157 \pm 8.5\%$ of unexposed cells ($n = 8$); 60 min $167 \pm 14.3\%$ of unexposed cells ($n = 8$), $p < 0.01$ and $p < 0.01$, respectively). This enhancing effect was lost with longer incubation periods (Table 2). The total concentration of 100 pM rhGM-CSF also significantly enhanced superoxide generation by adult granulocytes but only after a 60-min exposure. Unlike cord blood cells, significantly increased superoxide production persisted after a 120-min incubation with rhGM-CSF (60 min: $152 \pm 10\%$ of unexposed cells ($n = 5$); 120 min: $123 \pm 8\%$ of unexposed cells ($n = 5$); $p < 0.02$ and $p < 0.02$, respectively). Superoxide generation by cord blood granulocytes unexposed to rhGM-CSF was greater than unexposed adult cells, with a further increase after exposure to rhGM-CSF.

Effects of rhGM-CSF on granulocyte membrane depolarization

responses. In three to six experiments, cord blood granulocyte membrane depolarization responses were equivalent to those observed in paired adult control granulocytes for all fMLP concentrations tested (10^{-6} to 10^{-8} M). Exposure of either cord blood granulocytes or adult control granulocytes to 100 pM GM-CSF for 30, 60, or 120 min before fMLP stimulation did not alter the membrane depolarization responses of either cell type.

Effects of rhGM-CSF on granulocyte phagocytosis. Phagocytosis of *S. aureus* by cord blood granulocytes was equivalent to that of adult granulocytes (Fig. 3). Preincubation (30 min) of cord blood cells with 100 pM rhGM-CSF did not alter the numbers of granulocytes ingesting serum opsonized *S. aureus*.

Effects of rhGM-CSF on granulocyte bactericidal activity. Cord blood granulocyte bactericidal activity was equivalent to adult cell bactericidal activity (Table 3). Over a 5 log range of rhGM-CSF concentrations (10 nM–1 pM), no alterations in bacterial killing by cord blood granulocytes were observed.

DISCUSSION

Overwhelming sepsis and meningitis occurs in one to three infants/1000 live births and in up to 30% of premature infants (28). Mortality in this disease varies from 20 to 50%. The neonate's increased susceptibility to infection has been attributed to a number of abnormalities in its host defenses (11), including abnormalities in neonatal granulocyte function. Depressed locomotion toward chemoattractants (14–17), defective membrane depolarization responses compared to adult granulocytes (18), abnormal phagocytosis (29, 30), and depressed bactericidal activity (30) are among the abnormalities described. Oxygen metabolism is generally normal (31, 32), although stressed neonates have been reported to have diminished production of superoxide

Fig. 2. Representative image analysis results examining cord blood granulocyte locomotion. The horizontal axis shows the distance moved by granulocytes toward the stimulus identified at upper right of each panel. One pixel = 6μ . The vertical axis shows the density of cells at each pixel of distance. Arrows show mean distance migrated for control (closed arrow) and 1 pM GM-CSF exposed (open arrow) cord blood granulocytes.

anion (32). rhGM-CSF *in vitro* has been reported to enhance each of these functions in granulocytes from adults.

We found that *in vitro* exposure to rhGM-CSF enhanced cord blood granulocyte locomotion and superoxide generation but did not effect the other functions tested. One picomolar rhGM-CSF significantly enhanced leading front migration of cord blood granulocytes when either fMLP, activated serum or fMLP plus activated serum was used as a chemoattractant. This effect was not observed with adult granulocytes tested in the same chemotaxis assay system with *in vitro* exposure to the same rhGM-CSF (33).

Our data suggest that the effect of rhGM-CSF on cord blood granulocyte locomotion is complex. Multiple facets are altered depending on the concentration and mode of presentation of rhGM-CSF to the cell. As shown in this report and by others (10), rhGM-CSF is a weak chemoattractant; when presented in a non-gradient fashion, it should cause chemokinesis. We hypothesize that the enhancing effect on unstimulated locomotion seen at the 100 pM rhGM-CSF concentration is such a chemokinetic effect. Inasmuch as the direct chemoattractant/chemokinetic activity of rhGM-CSF is weak, the chemokinetic effect is obscured when stronger chemoattractants (such as fMLP) are presented as a gradient, resulting in chemotaxis. At high concentrations (10 nM), rhGM-CSF suppresses chemotaxis, consistent with its original designation as neutrophil migration inhibition factor from T cells (9). Suppression of adult PMN chemotactic locomotion *in vitro* has also been observed at this rhGM-CSF concentration (33). At low GM-CSF concentrations (1 pM), an enhancing effect on cord granulocyte chemotactic locomotion occurs. Thus, rhGM-CSF can mediate a variety of locomotive effects on cord blood granulocytes, depending on the concentration and mode of presentation of the hematopoietin. Similar dose-dependent, opposing effects on neonatal granulocyte locomotion have been reported with pentoxifylline (34), a methylxan-

Table 2. Effects of rh GM-CSF on granulocyte superoxide production

Incubation time with/without GM-CSF (min)	nmol $O_2/10^6$ granulocytes/15 min							
	Cord blood granulocytes 10^{-6} M fMLP				Adult granulocytes 10^{-6} M fMLP			
	- GM- CSF	+ GM- CSF	- GM- CSF	+ GM- CSF	- GM- CSF	+ GM- CSF	- GM- CSF	+ GM- CSF
0	(8) 10.7	1.2	11.8	1.5	(5) 5.6	1.1	5.9	0.7
30	(8) 11	2.7	17.3*	3.9	(5) 7.2	1.3	10.3	2.0
60	(8) 12.5	2.4	20.4*	3.9	(5) 7.8	1.1	12.1†	1.9
90	(3) 11.4	2.7	12.9	2.0	(5) 7.4	1.4	10.0†	1.0
120	(8) 7.6	1.1	8.8	1.6	(5) 7.6	1.0	9.3†	1.1

* $p < 0.01$.

† $p < 0.02$.

Table 1. Effects of recombinant human GM-CSF on polymorphonuclear leukocyte locomotion*

Locomotive stimulus	Cord PMN movement Adult PMN movement 100			Cord PMN + 1 pM GM-CSF movement Adult PMN movement 100			
	(n)	Mean	SE	(n)	Mean	SE	p
Hanks' balanced salt solution	(18)	73	7	(18)	84	9	NS
Activated serum	(18)	80	10	(18)	91	12	<0.025
fMLP (5×10^{-7} M)	(18)	68	6	(18)	80	8	<0.020
Activated serum + fMLP (5×10^{-7} M)	(17)	72	7	(17)	84	7	<0.010

* Statistical significance determined by paired sample *t*-test.

Fig. 3. The effect of rhGM-CSF on granulocyte phagocytosis of *S. aureus*. The vertical axis shows the percentage of granulocytes with one or more intracellular bacteria. The horizontal axis shows the duration of contact with *S. aureus* before exposure to lysostaphin. Each curve is the mean of four to five experiments.

Table 3. Effects of rhGM-CSF on granulocyte bactericidal activity*

Condition	% bacterial survival					
	20 min		45 min		90 min	
	Mean	SE	Mean	SE	Mean	SE
Adult cells	51	4	32	6	17	1
Cord Blood cells	56	3	39	8	17	6
Cord Blood cells + 10 nM GM-CSF	61	5	35	10	19	3
Cord Blood cells + 100 pM GM-CSF	55	9	36	9	17	5
Cord Blood cells + 1 pM GM-CSF	56	8	36	7	17	6

* Data shown are from three separate experiments using a granulocyte:bacteria ratio of 1:2.

thine drug capable of enhancing human cord blood granulocyte chemotaxis (35).

The distance moved by the leading front of granulocytes is typically the only parameter measured in an under agarose chemotaxis assay, a parameter that could be enhanced by increased locomotion of only a small subpopulation of cells. To determine if rhGM-CSF enhanced chemotaxis by effecting a subpopulation of cells or the whole cell population, we used an image analysis system to specifically examine the effects of rhGM-CSF exposure on two other parameters of locomotion: the mean distance migrated by the cell population and the area under the population distribution (which represents the numbers of cells responding to the stimulus). In the three experiments examined, when fMLP was the chemoattractant, 1 pM rhGM-CSF significantly enhanced both of these parameters, in addition to the leading front distance. It appears unlikely, then, that the observed effects of the hematopoietin are due only to stimulation or suppression of a small subpopulation of responsive cells. Although rhGM-CSF did not normalize the locomotion of cord blood granulocytes when compared to adult cells, these experiments do confirm that the locomotive defect present in cord blood granulocytes can be partially reversed by pharmacologic means, a fact only recently appreciated (35).

Superoxide anion generation by cord blood granulocytes was increased after exposure to 100 pM rhGM-CSF, as has been previously reported for adult granulocytes (6). Cord blood cells exhibited maximal stimulation after a 30- to 60-minute incubation with rhGM-CSF but no alteration in superoxide anion production compared to untreated cord cells after a 2-h incubation. In our study, adult granulocytes also demonstrated an increase in superoxide anion production after incubation with rhGM-CSF, confirming the previous work. However, we showed the effect of rhGM-CSF on adult cells was maximal after 60 min as opposed to the 120 min reported by others (6). The reason for the time discrepancy between studies is unclear.

Cord blood and adult granulocyte phagocytosis, bactericidal activity and membrane depolarization responses were unchanged after exposure to rhGM-CSF. Other reports (6, 36) have reported increased phagocytosis after rhGM-CSF exposure. In their experiments, preopsonized organisms or low concentrations of serum were used to demonstrate the enhancing effect. The use of 10% autologous serum in our assay system may have obscured small degrees of enhancement. We conclude that if GM-CSF does enhance phagocytosis and bactericidal activity, it is a minor effect under optimal conditions for phagocytosis and microbial killing. Cord granulocyte membrane depolarization, a function thought to possibly reflect receptor-ligand initiated signal transduction in the granulocyte, has previously been reported to be defective in neonatal cells (18). Using fMLP as the stimulus, we found that neonatal and adult granulocytes had equivalent responses and that rhGM-CSF did not affect these responses in either cell type.

Several mechanisms have been proposed to explain selected *in vitro* effects of rhGM-CSF on granulocytes. Weisbart et al. (37) suggest that certain effects are mediated through changes in the affinity state of fMLP receptors. High affinity fMLP receptors are related to enhanced chemotaxis whereas low affinity receptors appear to be associated with increased oxidative metabolism (38). These authors noted that after brief exposure to rhGM-CSF, adult granulocytes showed a rapid increase in high affinity receptors which temporally coincided with enhancement of chemotaxis by rhGM-CSF. Longer incubation was associated with a shift to low affinity receptors, and temporally coincided with enhancement of granulocyte oxygen metabolism. Although our studies only examine neonatal cell function and not fMLP receptor affinities, our results, showing enhancement of neonatal cell locomotion, are at least partially consistent with this hypothesis.

A second mechanism postulates that rhGM-CSF recruits a subpopulation of granulocytes that are unresponsive to fMLP before exposure to GM-CSF (39). Subpopulations of neonatal cells with different functional capacities have previously been demonstrated (40). Our results could be consistent with the recruitment of a large population of poorly responsive cord blood granulocytes into the fMLP responsive population. Further testing of this hypothesis is currently underway.

In conclusion, we have demonstrated that selected cord blood granulocyte functions are enhanced by *in vitro* exposure to rhGM-CSF. An animal model is currently being developed to determine the *in vivo* significance of these *in vitro* observations.

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