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Decreased Stimulated GM-CSF Production and GM-CSF Gene Expression but Normal Numbers of GM-CSF Receptors in Human Term Newborns Compared with Adults[1]

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ABSTRACT. We investigated cord and adult production of granulocyte-macrophage colony-stimulating factor (GM-CSF), expression of GM-CSF mRNA from unstimulated and activated mononuclear cells, and the affinity and presence of GM-CSF receptors on mature effector cells in an attempt to better understand the underlying pathophysiology of altered neonatal host defense. Utilizing ^{125}I-GM-CSF as a ligand, Scatchard analysis revealed the presence of a single class affinity GM-CSF receptor with similar binding characteristics on both cord and adult peripheral PMN (k_d = 44 and 39 pM) for adult and cord, respectively. Additionally, there was no significant difference in the number of GM-CSF receptors on cord *versus* adult neutrophils. Using a sandwich ELISA for measuring GM-CSF levels, we found nondetectable levels from supernatants of unstimulated cord and adult mononuclear cells and serum from cord and adult peripheral blood. However, there was a significant difference between cord and adult GM-CSF production from stimulated phytohemagglutinin and phorbol-12-myristate-6-acetate mononuclear cells ($p < 0.02$). Additionally, GM-CSF mRNA expression from activated cord mononuclear cells was significantly reduced after 6 h of stimulation compared with adults. Nuclear run-on experiments revealed no difference in transcriptional activation from activated cord and adult mononuclear cells. Actinomycin D transcriptional decay studies, however, demonstrated reduced GM-CSF half-life from activated cord *versus* adult mononuclear cells ($t_{1/2}$ 30 *versus* 100 min). These results suggest normal affinity and numbers of GM-CSF receptors on peripheral mature effector cells but decreased GM-CSF production and GM-CSF mRNA expression from activated cord *versus* adult mononuclear cells. Reduced GM-CSF mRNA expression and production may explain in part the difference in host defense between the newborn and the adult during states of increased demand. (*Pediatr Res* 30: 362–367, 1991)

Abbreviations

GM-CSF, granulocyte-macrophage colony-stimulating factor
CFU-GM, colony forming unit granulocyte-macrophage
CSF, colony-stimulating factor
MNC, mononuclear cell
PMN, polymorphonuclear cell
PMA, phorbol-12-myristate-6-acetate
PHA, phytohemagglutinin
rh, recombinant human

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Hematopoiesis in the preterm and term newborn is developmentally immature compared with the adult (1). Specific differences in myelopoiesis have been demonstrated in term newborn rats compared with adult animals. Reduced neonatal rat myeloid progenitor pools, accelerated myeloid progenitor proliferative rates, and decreased total body neutrophil storage pools all predispose the newborn rat to depletion of mature effector neutrophils and a tendency to develop neutropenia during states of increased demand or overwhelming bacterial sepsis (2–4). During experimental sepsis (states of increased demand), adult animals increase their CFU-GM pool two to three times greater than baseline and increase their proliferative rate to approximately 75% of maximal capacity. In contrast, however, term newborns under the same conditions decrease their already reduced CFU-GM pool by almost 50% and fail to increase their already maximal proliferative myeloid pool, remaining at 75–80% of capacity (5, 6). Additionally and most importantly, neonatal rats also deplete their already reduced neutrophil storage pool reserves by almost 80–90%, compared with a decline of only 25–33% in adult rats (6, 7).

Recently, it has been demonstrated that cellular proliferation, maturation, and differentiation of hematopoietic progenitor cells and the regulation of hematopoiesis are dependent in part on the continuous and/or intermittent supply of highly specific hematopoietic growth factors (CSF) (8–11). CSF stimulate proliferation of bone marrow myeloid progenitor cells, induce the release of mature effector neutrophil storage pools, and enhance mature neutrophil effector function. CSF may, in fact, be the major regulator of increased myeloid cell production during states of increased demand. GM-CSF, first purified to homogeneity from a medium conditioned by a human T lymphotrophic virus (HTLV II)-infected T lymphoblastoid cell line (MO), is one such CSF that stimulates the formation of granulocyte, macrophage, and eosinophil colonies in human bone marrow cell culture (12, 13). GM-CSF additionally primes adult neutrophils for enhanced oxidative metabolism, chemotaxis, and phagocytosis; induces expression of surface active adhesion glycoproteins such as C2bi; and enhances antibody-dependent cytotoxicity

(14–18). GM-CSF has recently been utilized as adjuvant therapy in the treatment of a number of clinical conditions including aplastic anemia, AIDS, bone marrow transplantation, and myelodysplasia (19).

Recently, we have demonstrated that rhGM-CSF primes neonatal granulocytes for enhanced oxidative metabolism, chemotaxis, bacterial killing, C3bi expression, and adherence (20, 21). Murine GM-CSF has also been noted to enhance neonatal rat myelopoiesis and have some protective activity against bacterial infection in the newborn rat (22, 23). In an attempt to understand the differences in host defense between the newborn and the adult and the potential usefulness of exogenous GM-CSF administration in the neonatal period, we investigated the constitutive and stimulated production of GM-CSF from adult and newborn MNC, expression of GM-CSF mRNA under similar conditions, and the affinity and number of GM-CSF receptors on mature neonatal and adult neutrophil effector cells.

MATERIALS AND METHODS

Isolation of human PMN and MNC. 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 scheduled cesarean section. Adult or cord red blood cells and neutrophils were allowed to sediment after the addition of 6% Dextran-70 in 0.9% saline (Baxter Laboratories, Irvine, CA). The leukocyte-enriched plasma was collected, layered on a Ficoll-Hypaque gradient (density = 1.077) (Sigma Chemical Co., St. Louis, MO), and centrifuged for 30 min. PMN were recovered in the cell pellet, and any remaining erythrocytes were hemolyzed by hypotonic lysis. MNC were recovered in the liquid interface and were then suspended for culture in RPMI 1640 (Gibco, Grand Island, NY) with 10% human AB serum (Sigma), glutamine, and penicillin/streptomycin. PMN and MNC isolated by this density gradient separation were purified to greater than 98% homogeneity, and cell viability as measured by trypan blue exclusion was more than 99% (adult: 86 ± 4.0% lymphocytes and 7.2 ± 3.0% monocytes; cord: 82 ± 8.0% lymphocytes and 8.8 ± 4.0% monocytes).

PMN were used in the following assays within 2 h of collection. MNC (1.0×10^6 cells/mL) were stimulated in culture with PMA (Sigma) (20 ng/mL), and PHA (2 μg/mL) (Gibco). Supernatants were harvested at 24–72 h and assayed for GM-CSF levels.

Binding of iodinated GM-CSF to neutrophils. Isolated neutrophils were suspended in a binding medium of Iscove's modified Dulbecco's medium (Gibco) with 25 mM *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid, and BSA (2 mg/mL) (pH 7.4). Cells (4×10^6) were suspended in binding medium containing various concentrations of ^{125}I-labeled GM-CSF (DuPont/New England Nuclear), with or without a 100-fold excess of unlabeled GM-CSF, and incubated for 2 h at 23°C. After incubation, the cells were resuspended and transferred onto an ice-cold mixture of 75% FCS in a binding medium (24). The cells were centrifuged for 2 min, supernatant was aspirated, and the radioactivity of the pellets was counted in an LKB universal gamma counter (Pharmacia, Milwaukee, WI). Specific binding was defined as the amount of binding blocked by competition with a 100-fold excess unlabeled GM-CSF. Data was analyzed by weighted nonlinear least squares curve-fitting developed by Munson and Rodbard (25).

GM-CSF levels. GM-CSF concentrations from MNC culture supernatants and healthy donor serum samples were measured by a sandwich ELISA adopted from Cebon *et al.* (26). Microtiter plates were coated with the mouse antihuman GM-CSF MAb (Genzyme, Boston, MA) (200 ng/40 μL) in PBS (16 h at 20°C) and then blocked with PBS with 1% (wt/vol) BSA (Sigma) for 6 h at 20°C. The plates were rinsed with water, blotted dry, and stored at −20°C until use. After the plates were thawed, a 150 μL sample was incubated at 4°C for 16 h. The plates were then washed with washing buffer (PBS with 0.1% vol/vol Tween 20). Rabbit antihuman GM-CSF polyclonal antibody (Genzyme) was diluted 1:1500 in EDB (PBS with 0.2% Tween 20 vol/vol and 0.1% BSA wt/vol) and incubated for 3 h at 20°C. Bound rabbit antibody was detected using biotinylated antirabbit Ig from donkey (Amersham, Chicago, IL) at 1:500 vol/vol in EDB. After washing the plates, streptavidin-biotinylated horseradish peroxidase complex (Amersham) diluted 1:1000 in EDB was incubated for 15 min. Finally, 150 μL of 3, 3′, 5, 5′-tetramethylbenzidine solution with hydrogen peroxide (TMB Peroxidase EIA kit; Bio-Rad Laboratories, Richmond, CA) were added and the reaction was stopped at 30 min with 1 N H_2SO_4. Optical density was measured at 450 nm with Bio-Rad EIA reader. RhGM-CSF (Amgen, Thousand Oaks, CA) was used as the standard for the ELISA (5 pg/mL to 50 ng/mL). Patient serum samples were diluted with PBS 1% BSA wt/vol before analysis and rhGM-CSF levels were determined using a standard curve of predetermined concentrations of rhGM-CSF in human serum.

RNA isolation and Northern blotting. Total RNA was extracted as previously described by Chomczynski *et al.* (27) and electrophoresed on a 1% agarose, 5% formaldehyde gel. The samples were heated in 40% formamide, 14% formaldehyde at 65°C for 15 min and then cooled. To ensure that an equal amount of RNA was loaded in each lane, 1 μg ethidium bromide was added to each sample before loading. RNA was transferred to nitrocellulose and baked 2 h. Hybridization was done at 42°C in 50% formamide, 5 × sodium chloride, sodium citrate (SSC), 1 × Denhardt's, 50 mM sodium phosphate (pH 6.5), 0.1% SDS, 250 μg/mL salmon sperm DNA, and 10% dextran sulfate and probed with ^{32}P-labeled GM-CSF cDNA (B1H1 from ATCC). Filters were washed to a stringency of 0.3 × SSC at 65°C and exposed to Kodak XAR-5 film.

Nuclear run-on transcription assays. A total of 10^8 MNC were stimulated for 6 h with PMA 20 ng/mL, PHA 2 μg/mL. Nuclei isolation and nuclear run-ons were done using slight modifications of previously described techniques (28). The cells were washed in PBS, lysed in 4 mL lysis buffer [10 mM Tris (pH 7.4), 10 mM NaCl, 3 mM $MgCl_2$, 0.5% NP-40] on ice for 5 min, and centrifuged for 5 min at 1500 rpm (4°C) in a Beckman TJ-6 centrifuge to pellet the nuclei. The nuclei were washed in lysis buffer, then resuspended in 100 μL storage buffer (40% glycerol, 50 mM Tris, 5 mM $MgCl_2$, 0.1 mM EDTA) and kept at −70°C until use. To label the nuclear RNA, 50 μL of elongation mix [20 mM Tris (pH 8.0), 10 mM $MgCl_2$, 100 mM KCl, 10 mM DTT]; 5 μL each of 10 mM ATP, guanosine triphosphate, and cytidine triphosphate; 5 μL RNAsin (Promega, Madison, WI); 20 μL ^{32}P-uridine triphosphate (Amersham; 3000 Ci/mmol); and 25 μL H_2O were added to the nuclei and heated at 30°C for 30 min. The reaction was terminated by the addition of 40 IU RNase-free DNase and further incubated at 30°C for 10 min. Ten microliters of stop buffer [10% Sarcocyl, 100 mM EDTA, 100 mM Tris (pH 7.6), 1 mg/mL Proteinase K] were added and heated at 40°C for 30 min. Fifteen microliters yeast transfer RNA was added as carrier, followed by three phenol/chloroform/isoamyl extractions, and precipitated with ethanol. Four micrograms of linearized denatured single-stranded plasmid DNA was loaded per well onto nitrocellulose in a slot blot apparatus (Schleicher & Schuell, Inc., Keene, NH). The plasmid DNA coding for GM-CSF was a 5.2-kb *Hin*dIII fragment of B1H1 (ATCC) and the β-actin was a 2-kb *Bam*HI fragment (29). The filter was baked 2 h, hybridized with the ^{32}P-labeled RNA for 36 h in 50% formamide at 42°C, washed to 0.3 × SSC at 65°C, then exposed to Kodak XAR-5 x-ray film.

Half-life. A total of 5×10^6 MNC were stimulated for 6 h with PMA 20 ng/mL, PHA 2 μg/mL. To block transcription, actinomycin D was added to the culture medium at a final concentration of 5 μg/mL (30). Cells were harvested at indicated times (0–90 min) and RNA was isolated. The mRNA was detected by Northern hybridization using the B1H1 GM-CSF probe.

RESULTS

Cord and adult human neutrophils were isolated by Ficoll-Hypaque density centrifugation. Using ^{125}I-GM-CSF, we determined the equilibrium binding of this ligand to both purified cord and adult human neutrophils. Neutrophils were exposed to iodinated GM-CSF (≤14 000 cpm) in the presence or absence of 100-fold excess of unlabeled ligand for up to 2 h at 23°C. As shown in Figure 1, the binding curve and Scatchard analysis of adult neutrophil GM-CSF receptors revealed a single class high-affinity receptor with a k_d of approximately 44 pM. Similarly, in Figure 2, the equilibrium curve and Scatchard analysis demonstrated cord neutrophils to have a single high-affinity GM-CSF receptor and a similar k_d of 30 pM. Using ^{125}I-GM-CSF as a ligand, we found a similar number of GM-CSF receptors on cord neutrophils *versus* adult neutrophils (444 ± 66.2 *versus* 658 ± 138 sites/cell, respectively) (p = NS).

Cord and adult MNC were also isolated by Ficoll-Hypaque density centrifugation. Adult and cord serum were additionally separated. Using an ELISA consisting of a murine antihuman GM-CSF antibody, a rabbit antihuman GM-CSF antibody, and a donkey antirabbit antibody, we measured GM-CSF levels from circulating serum, constitutive supernatants, and stimulated supernatants from cord and adult MNC. Serum levels from both cord and adult MNC cells were both nondetectable (<10 pg/mL) (n = 4). Similarly, constitutive supernatant levels of GM-CSF from cord and adult MNC also demonstrated nondetectable GM-CSF levels (<10 pg/mL).

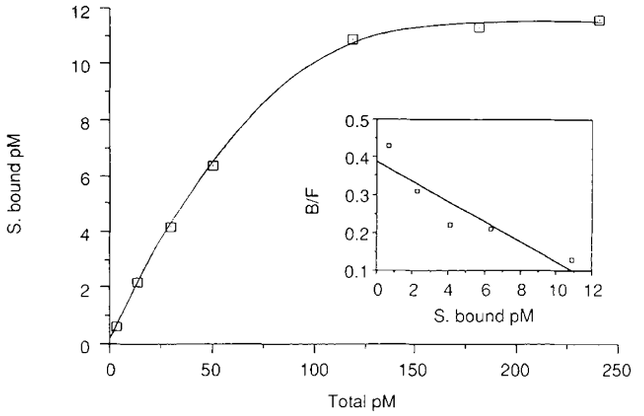

Fig. 1. Specific binding of ^{125}I-labeled GM-CSF to neutrophils from adult peripheral blood. The amount of nonspecific binding not blocked by competition with a 100-fold excess of unlabeled GM-CSF was subtracted from total binding to give the values represented here (n = 2). *Insert,* Scatchard analysis of these equilibrium binding data (k_d = 44 pM).

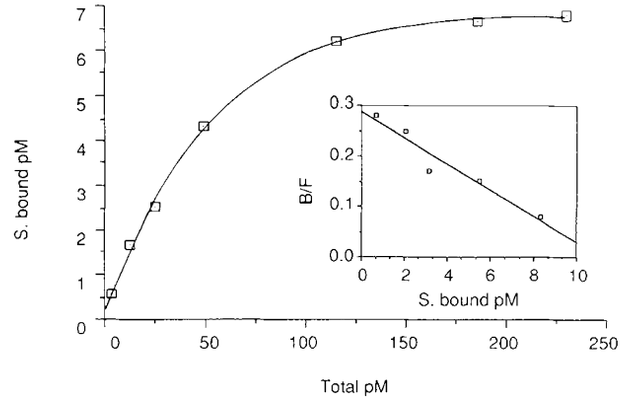

Fig. 2. Specific binding of ^{125}I-labeled GM-CSF to neutrophils from cord blood. The amount of nonspecific binding not blocked by competition with a 100-fold excess of unlabeled GM-CSF was subtracted from total binding to give the values represented here (n = 2). *Insert,* Scatchard analysis of these equilibrium binding data (k_d = 39 pM).

Cord and adult MNC were stimulated with PMA 20 ng/mL and PHA 2 μg/mL for 0–72 h, and supernatants were analyzed for GM-CSF production. Maximal stimulation from adult MNC occurred within 48–72 h after stimulation. There appeared to be little difference in GM-CSF production between 24 and 72 h from stimulated cord MNC. Strikingly, however, there was a marked and significant difference in GM-CSF production between cord and adult stimulated MNC (4.0 ± 1.1 *versus* 0.57 ± 0.16 ng/mL, adult *versus* cord; $p < 0.02$) (72 h).

To determine if decreased GM-CSF production from stimulated cord MNC was secondary to decreased gene expression, cord and adult MNC unstimulated and stimulated, were evaluated for GM-CSF mRNA expression. Using the GM-CSF probe B1H1 and a positive and negative control cell line (MoT and A172, respectively), GM-CSF mRNA transcripts were detected by Northern blot hybridization and analyzed by densitometry of autoradiographs. Unstimulated cord and adult MNC GM-CSF mRNA transcripts were barely detectable in both cell populations (time zero) (Fig. 3). However, after 6 h of stimulation with PHA and PMA, cord MNC expressed significantly decreased GM-CSF mRNA transcripts (Fig. 3). By 24 h however, there was normal expression of cord GM-CSF mRNA transcript (Fig. 3).

To examine the difference between cord and adult GM-CSF mRNA production, we performed nuclear run-on transcription experiments after PHA and PMA treatment. After transcriptional activation, nuclei from untreated and PHA- and PMA-treated cord and adult MNC were isolated and the RNA was hybridized to GM-CSF cDNA. After 6 h of stimulation, there was no significant difference in transcriptional activation between cord and adult MNC GM-CSF transcription (Fig. 4). We

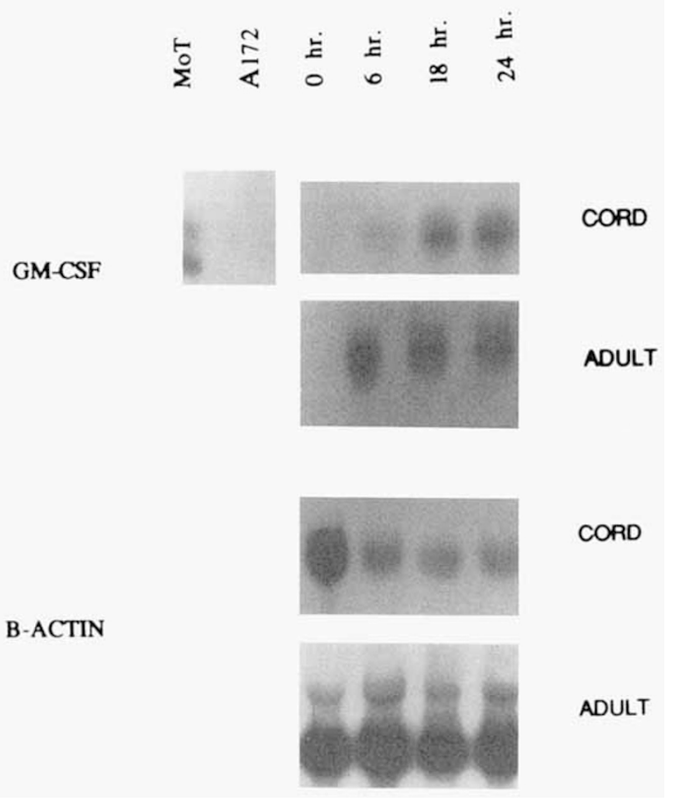

Fig. 3. GM-CSF mRNA levels from unstimulated and stimulated MNC from cord and adult peripheral blood. Cells were isolated and stimulated with PMA 20 ng/mL and PHA 2 μg/mL, and RNA was extracted (B1H1). The Northern blot was hybridized with ^{32}P-labeled GM-CSF probe. *MoT* is a human T cell leukemia cell line serving as a positive control for GM-CSF. *A172* is a glioblastoma cell line serving as a negative control for GM-CSF (n = 4).

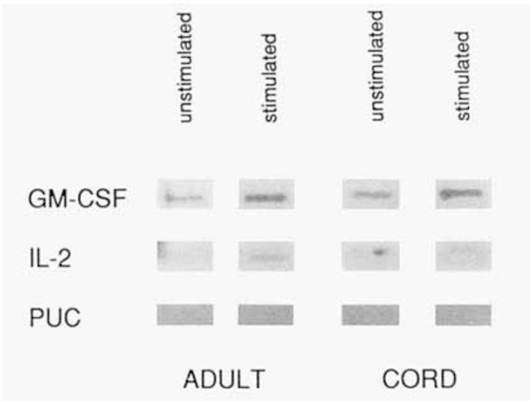

Fig. 4. Nuclear run-on analysis of GM-CSF transcription rates from MNC stimulated with PMA/PHA from both adult and cord peripheral blood. An equal number of counts were hybridized to filters containing the indicated probes ($n = 4$) *PUC*, plasmid controls.

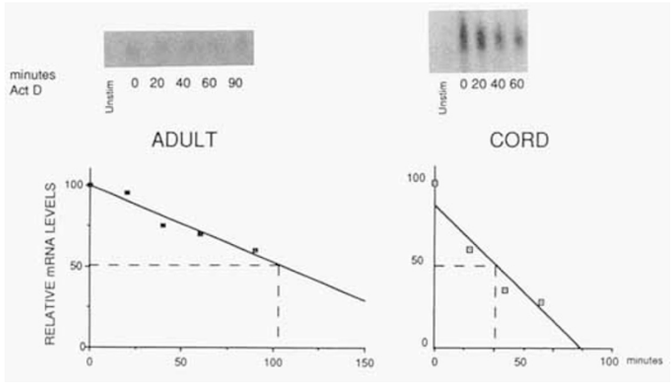

Fig. 5. Northern blot analysis of the half-life of GM-CSF mRNA in stimulated adult and cord MNC. Actinomycin D (5 μg/mL) was added for the indicated times to cells stimulated for 6 h with 20 ng/mL PMA and 2 μg/mL PHA. Total cellular RNA was isolated and hybridized to the ^{32}P-labeled GM-CSF as described ($n = 4$).

additionally determined the decay of GM-CSF mRNA by inhibiting RNA synthesis with actinomycin D. RNA was extracted from unstimulated and stimulated cord and adult MNC and incubated with actinomycin D (5 μg/mL) and GM-CSF mRNA was analyzed by Northern blot hybridization and quantitated by densitometry. After 6 h of stimulation with PMA and PHA, the transcriptional decay of GM-CSF mRNA was significantly different between cord MNC and adult MNC. The estimated transcriptional half-life decay of GM-CSF mRNA from cord MNC was approximately 30 min compared with 100 min from adult MNC (Fig. 5).

DISCUSSION

Peripheral neutropenia is a hallmark finding during overwhelming bacterial sepsis in the newborn and is associated with a poor prognosis (31–34). A decrease in total body myeloid progenitor cells (CFU-GM), near-maximal proliferative capacity of myeloid progenitor cells, an accelerated egress of mature neutrophil storage pools from the bone marrow reserve, and accelerated neutrophil use during infection all predispose the human neonate to significant neutropenia during bacterial sepsis (3–7). The true incidence of severe neutrophil storage pool depletion (<7%) (PMN + band + metamylocytes) associated with neonatal neutropenia and sepsis is probably less than 15% (35, 36). However, the presence of severe (<7%) neutrophil storage pool depletion in neonates with sepsis may not be the only indicator of a poor prognosis in this patient population. Recently, we demonstrated that even a mild or moderate decrease in the neutrophil storage pool may be associated with an increase in mortality during overwhelming bacterial sepsis (37).

GM-CSF, a hematopoietic growth factor, stimulates bone marrow progenitor cells and influences the growth and proliferation of CFU-GM and CFU-eosinophil, inducing an increase in neutrophils, monocytes, and eosinophils in the peripheral blood (12, 13). GM-CSF has been demonstrated to induce peripheral neutrophilia both secondary to release and egress of neutrophil storage pools cells within the first 24 h of administration and a sustained peripheral neutrophilia resulting from stimulation of early myeloid progenitor cells and, additionally, has been noted to correct *in vitro* mature effector neutrophil physiologic dysfunction (38–40). We have recently demonstrated that human GM-CSF also primes and stimulates neonatal neutrophils for enhanced functional activity (20, 21). Broxmeyer *et al.* (41) have also demonstrated that term cord blood myeloid progenitor cells, *i.e.* CFU-GM, respond similarly to adult bone marrow CFU-GM during *in vitro* human GM-CSF stimulation. Laver *et al.* (42), using a bioassay, have even demonstrated increased circulating plasma levels of GM-CSF from term umbilical cord blood compared with normal circulating adult plasma levels.

Our study was designed to evaluate the affinity and number of GM-CSF receptors on mature effector neutrophils from both cord and adult cells and to additionally determine constitutive and stimulated GM-CSF production from both cord and adult MNC. Using ^{125}I-GM-CSF as a ligand, we were able to demonstrate similar binding characteristics and a single class affinity receptor for GM-CSF. Additionally, we have demonstrated that the number of GM-CSF receptors on peripheral mature effector neutrophils is similar from both cord and adult peripheral blood. Our affinity studies and the number of GM-CSF receptors on adult neutrophils are similar to those found by a number of other investigators (43, 44). It remains to be seen, however, whether the number and/or affinity of GM-CSF receptors is similar or different between cord and adult bone marrow myeloid progenitor cells.

GM-CSF production, however, was markedly different from activated cord MNC compared with activated adult MNC. During *in vitro* stimulation with PHA and PMA, activated cord MNC produced almost 16% of GM-CSF compared with similar activated adult MNC. This is in stark contrast to constitutive GM-CSF levels from supernatants of unstimulated cord and adult MNC and circulating serum levels, which were barely detectable using our ELISA. This differs from the study by Laver *et al.* (42), who demonstrated increased circulating plasma levels of GM-CSF in cord blood compared to adult blood. The difference in our constitutive levels compared with Laver's are probably 2-fold. In the study by Laver *et al.* (42), they chose to measure plasma GM-CSF levels by using a bioassay (human cell line TALL 101). The growth in this bioassay, however, may be influenced by other growth factors and may lead to different conclusions. In fact, as reported in Laver's study (42), when the authors coincubated with MAb to GM-CSF (anti-GM-CSF), they demonstrated an 11% residual bioactivity from their cord samples, probably secondary to the presence of other growth factors. Additionally, the difference in the results from our stimulated mononuclear studies may include the lack of contribution of endothelial cells or fibroblasts to GM-CSF production. Therefore, total GM-CSF production could be estimated from this study. Lastly, this study did not evaluate whether the decrease in GM-CSF production from cord MNC occurs secondary to a decrease per cell or a decrease in a subpopulation of GM-CSF producing MNC.

Our data additionally suggest that decreased GM-CSF production from activated cord MNC compared with adults may in part be secondary to decreased GM-CSF mRNA expression. After a 6-h incubation with PHA and PMA, cord MNC expressed significantly reduced GM-CSF mRNA compared with similarly stimulated adult MNC. In an attempt to elucidate the mechanism for decreased GM-CSF mRNA expression after 6 h of stimulation

from cord MNC compared with adults, we studied transcriptional activation with nuclear run-on studies and transcriptional decay with actinomycin D inhibition studies. The nuclear run-on studies suggested no difference in transcriptional activation between cord and adult MNC relative to GM-CSF expression. However, our actinomycin D half-life studies suggested decreased stabilization of GM-CSF mRNA from 6-h stimulated cord MNC compared with adult cells. This reduced stabilization of GM-CSF mRNA from stimulated cord MNC may account in part for the decreased amount of GM-CSF mRNA and GM-CSF production during states of activation.

Our data suggesting decreased GM-CSF production and decreased GM-CSF mRNA expression from cord MNC are consistent with that of other investigations evaluating the production and activation of other cytokines from newborn MNC. Specifically, tumor necrosis factor-α appears to be decreased from stimulated neonatal MNC and may be secondary to decreased tumor necrosis factor-α mRNA expression (45, 46). Similarly, recent studies have demonstrated decreased γ-interferon production and reduced γ-interferon mRNA from stimulated human neonatal MNC (47, 48).

The control of GM-CSF production by adult MNC is secondary to a series of transcriptional and posttranscriptional regulatory processes. Nimer *et al.* (49) demonstrated that the genomic region responsible for the expression of GM-CSF mRNA from activated adult T lymphocytes probably occurs within a 90-bp region containing 53 nucleotides upstream and 37 nucleotides downstream from the start site of GM-CSF transcription. Using the technique of DNAase I footprinting, Gasson *et al.* (50) demonstrated that nuclear extracts from MLA-144 cells stimulated with PHA and TPA were bound to specific regions from this GM-CSF promoter region. Additionally, the GM-CSF gene has been demonstrated to be AT-rich in the 3′ untranslated segment, a finding similar to that in other cellular genes including c-*myc* and c-*fos*. This AT-rich region has been shown to have a destabilizing effect on mRNA production by recombinant constructs and, therefore, may play an important role in GM-CSF mRNA decay (51). Further investigations are planned to examine the difference between cord and adult GM-CSF mRNA stability and GM-CSF gene transcription from activated MNC.

During states of increased demand, quantitative deficiencies in neonatal myeloid progenitor activity and decreased availability of mature effector neutrophils have predisposed the newborn to a high mortality rate during overwhelming bacterial sepsis. Previous prospective and randomized trials using adult PMN transfusions *versus* standard supportive care have suggested a possible therapeutic benefit of using readily available and functionally active adult granulocytes to reduce the morbidity and mortality associated with bacterial sepsis in the newborn (37, 52–54). The benefits of granulocyte transfusions in septic neutropenic neonates must be carefully evaluated against the possible side effects associated with such transfusions, and additional questions still remain regarding the future role of such therapy. Therefore, other therapeutic measures designed to maintain high circulating neutrophil counts during overwhelming bacterial sepsis in the newborn must be explored. Recently, we demonstrated that prophylactic and prolonged G-CSF administration to newborn rats was protective in reducing the high mortality rate associated with experimental group B streptococcal sepsis (55). Additionally, murine GM-CSF has been noted to enhance neonatal rat myelopoiesis and reduce the mortality rate associated with experimental bacterial infection in the newborn rat (22, 23).

In summary, this study has demonstrated that stimulated cord MNC manifest decreased GM-CSF production and GM-CSF mRNA expression compared with similar stimulated adult MNC. Additionally, circulating GM-CSF serum levels and constitutive GM-CSF supernatants from unstimulated MNC are barely detectable from both cord and adults. The affinity and binding and the number of GM-CSF receptors on cord mature neutrophil effector cells are similar to those demonstrated in similar populations of adult cells. The decrease in GM-CSF production and gene expression may be secondary to decreased stability of cord mononuclear GM-CSF mRNA. Reduced stability of GM-CSF mRNA from cord MNC may account, in part, for altered host defense that has been previously demonstrated in the newborn. The demonstration of normal levels of GM-CSF receptors on peripheral cord effector cells, the *in vitro* functional activation of circulating cord mature neutrophil effector cells by GM-CSF, and the previous demonstration that murine GM-CSF modulates neonatal rat host defense against experimental bacterial infection all suggest the possible future role of exogenous rhGM-CSF to modulate neonatal human host defense.

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