Therapeutic Implications of Dysregulated Colony-Stimulating Factor Expression in Neonates

By Mitchell S. Cairo

Significant developmental immaturities in neonatal host defense predispose the neonates to an increased mortality rate during overwhelming bacterial sepsis. Dysregulation of neonatal hematopoiesis contributes to the development of peripheral cytopenias, including neutropenia and thrombocytopenia, during states of increased demand. Reduced neonatal rat myeloid progenitor pools, accelerated myeloid progenitor proliferative rates, and decreased total body neutrophil storage pools (postmitotic pool) 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. During experimental sepsis, adult animals increase their colony-forming unit granulocyte-macrophage (CFU-GM) pool 2 to 3 times greater than baseline and increase their proliferative rate to approximately 75% of maximal capacity. In contrast, term newborn animals, under the same conditions, decrease their already-reduced CFU-GM pool by almost 50% and fail to increase their CFU-GM proliferative rate, remaining at 75% to 80% of capacity. Most importantly, during experimental sepsis, neonatal rats also deplete their already-reduced neutrophil storage pool reserves by almost 80% to 90% compared with a decline of only 25% to 33% in adult rats.

In the human newborn, peripheral neutropenia is a hallmark finding during overwhelming bacterial sepsis and is associated with a poor prognosis. The incidence of severe neutrophil storage pool depletion (<7% [PMNs + bands + metamyelocytes]) associated with neonatal sepsis has ranged between 6% and 62%. Adult donor neutrophil transfusions have been used as adjuvant therapy in the treatment of newborn infants with neutropenia and overwhelming bacterial sepsis. We recently summarized our prospective randomized trial of neutrophil transfusions versus supportive care with or without intravenous gammaglobulin. In the group of newborn infants receiving neutrophil transfusions, there were 43 of 45 survivors (96%) compared with only 20 of 30 (66%) survivors in the supportive care and/or intravenous Ig group (P < .002). However, a number of questions still remain to be answered regarding the future role of adult donor neutrophil transfusions in the treatment of septic neutropenic newborn infants. New methods of treatment that induce both an increase in the neonatal circulating absolute neutrophil count and the bone marrow neutrophil storage pool during overwhelming bacterial sepsis need to be developed to decrease the morbidity and mortality associated with this disorder.

During stress or states of increased demand for phagocytic immunity, even normal numbers of circulating neutrophils may be insufficient. Numerous in vitro abnormalities have been shown in neonatal neutrophils, especially during times of stress or overwhelming bacterial infection. Specific defects in the neonatal neutrophil have included decreased deformability, chemotaxis, phagocytosis, C3bi receptor expression, adherence, bacterial killing, and oxidative metabolism.

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. To determine the mechanisms associated with neutropenia during states of increased demand in the newborn, several investigators have recently compared the production of colony-stimulating factors (CSFs) from unstimulated and activated neonatal versus adult peripheral blood mononuclear cells (MNC). Our laboratory initially showed a significant reduction in granulocyte-macrophage colony-stimulating factor (GM-CSF) production and GM-CSF mRNA expression from activated term newborn versus adult MNC. Despite decreased GM-CSF production and gene expression in activated term newborn MNC, mature effector neutrophils expressed a similar GM-CSF receptor number and affinity between term newborns and adults. We subsequently showed a similar reduction in both G-CSF and IL-3 production and gene expression from activated term newborn versus adult MNC. Similar to GM-CSF, G-CSF receptors were equal both in number and affinity on term newborn versus adult mature effector neutrophils.

In this issue of Blood, Schibler et al extended these ob-
servations and pursued the mechanisms associated with dysregulation of granulocyte colony-stimulating factor (G-CSF) expression and neonatal myelopoiesis. To eliminate the possibility of altered lymphocyte function and defective lymphokine production as a contributing factor to dysregulation of G-CSF expression, Schibler et al isolated and purified monocytes by panning with a cocktail of monoclonal antibodies to achieve greater than 90% pure monocytes from preterm, term, and adult peripheral blood. Using a population of pure monocytes, they were able to show a significant decrease in G-CSF production from supernatants of monocytes stimulated with interleukin-1α (IL-1α) in both preterm and term newborn compared with adult cells. Furthermore, after IL-1α activation, G-CSF mRNA expression was significantly reduced from both term and preterm versus adult monocytes. However, after lipopolysaccharide (LPS) activation and using competitive reverse transcriptase polymerase chain reaction (PCR), there was only a significant reduction in G-CSF mRNA expression from activated preterm compared with term or adult MNC.

To eliminate the possibility of decreased responsiveness by granulocyte progenitor cells to G-CSF as a mechanism of impaired newborn myelopoiesis, Schibler et al studied CFU-GM colony formation stimulated by recombinant G-CSF and found no significant difference between preterm, term, and adult progenitor cells. Additionally, there was no significant difference in circulating G-CSF levels between healthy preterm or term newborns and adults. However, there was a significant increase in circulating G-CSF levels in noninfected neutropenic adults compared with neutropenic neonates. These data suggest that newborn monocytes are defective in G-CSF expression during activated states and that, during neonatal neutropenic conditions, circulating G-CSF levels do not increase significantly above baseline.

We have recently extended our studies to determine whether the production of other hematopoietic growth factors that act at an earlier stage of hematopoiesis than GM-CSF or G-CSF are impaired in the newborn compared with the adult. Stem cell factor (SCF), a multipotent cytokine otherwise known as mast cell growth factor, Steel factor, or Kit ligand, is synergistic with a number of other cytokines to induce multilineage hematopoiesis. We have shown increased gene expression of SCF and its receptor, c-kit, from human umbilical vein endothelial cells compared with adult aortic endothelial cells. IL-11, an early acting cytokine isolated from primate stromal cells, has also been shown to be synergistic with a number of other cytokines to induce multilineage hematopoiesis. Similar to our studies with SCF, we have recently shown that activated endothelial cells and fibroblasts express significantly higher IL-11 mRNA levels from neonatal versus adult sources. These studies have suggested disparate regulation of cytokines in the newborn, with increased SCF and IL-11 and decreased GM-CSF, G-CSF, and IL-3 expression during activated conditions (Table 1).

To further elucidate the mechanisms associated with dysregulation of cytokine expression in the newborn, studi-
ies have been performed to determine whether this downregulation of CSF gene expression is secondary to decreased transcriptional activity of the corresponding genes. We have recently found the transcriptional rates of GM-CSF, G-CSF, and IL-3 to be similar from activated cord versus adult mononuclear cells using nuclear run-off assays. Therefore, alterations in posttranscriptional events such as mRNA stability could account for the difference between newborn and adult CSF expression. Using actinomycin D transcriptional decay studies, we have shown reduced GM-CSF half-life from activated cord versus adult MNC. Many cytokines and protooncogenes, including GM-CSF, G-CSF, and IL-3, share conserved AU-rich sequences in the 3′ untranslated region of their messages. Specifically, the 3′ untranslated regions of GM-CSF, G-CSF, and IL-3 contain repeated AUUUA motifs in an AU-rich context, which are known to mediate selective mRNA degradation in vitro and may cause mRNA instability of the above CSFs in vivo. Labile proteins involved in mRNA degradation, interacting with specific mRNA sequences (eg, AUUUA motifs), may regulate AU-rich mRNA decay. Alteration in the production and/or biological activity of these mRNA binding proteins may contribute to dysregulation of the AU-rich mRNAs coding for GM-CSF, G-CSF, and IL-3.

Alternatively, increased expression of negative hematopoietic regulators may contribute to the immaturity of neonatal hematopoiesis. The expression of two such negative regulators, transforming growth factor-β1 (TGF-β1) and macrophage inflammatory protein-1α (MIP-1α) in activated newborn versus adult MNC were examined. Similar to GM-CSF, G-CSF, and IL-3, newborn MNC expressed significantly reduced transcripts of TGF-β1 and MIP-1α during states of activation. Therefore, increased expression of negative hematopoietic regulators probably does not contribute significantly to impaired myelopoiesis and thrombopoiesis in the newborn.

These and other studies have suggested that endogenous G-CSF production is an important positive regulator of granulopoiesis during states of increased demand. G-CSF production is inversely correlated with the severity of neutropenia postmyeloablative therapy after allogeneic and autologous bone marrow transplantation. The absolute neutrophil count (ANC) was less than 200/μL, the endogenous G-CSF level was elevated 10-fold compared with the level when the ANC ranged between 200 and 500/μL. Additionally, Kawakami et al examined the endogenous levels of G-CSF before, during, and after acute sepsis. The level of G-CSF during the acute stage of infection was elevated up to 100-fold in adults. These data suggest that the inability of newborn infants to significantly upregulate G-CSF expression during states of increased demand results in decreased circulating G-CSF levels and impaired neutrophil production during overwhelming bacterial sepsis.

To determine whether exogenous administration of rhG-CSF could overcome some of the immaturities of neonatal myelopoiesis, we have examined the effect of both single and continuous administration of rhG-CSF in newborn rats during normal and experimental septic conditions. A single-pulse administration of rhG-CSF to 1-day-old neonatal rats resulted in a significant increase in peripheral neutrophilia and a reduction in mortality during experimental group B streptococcal infection. Seven-day administration of rhG-CSF in newborn rats induced a significant increase in peripheral neutrophilia and bone marrow neutrophil storage pools, and a significant reduction in the mortality rate during experimental group B streptococcal infection. Simultaneous administration of early acting CSFs such as SCF and IL-11 + G-CSF also results in a significant induction of peripheral neutrophilia and bone marrow myeloid pools in term newborn rats. To determine the toxicity and biologic efficacy of rhG-CSF in human newborn infants, we recently conducted a phase I trial of rhG-CSF in infants between 26 and 40 weeks of gestation with presumed sepsis. rhG-CSF was found to be well tolerated and induced a significant increase in both peripheral and bone marrow neutrophil pools.

The study by Schibler et al along with our results, suggest that the high incidence of neutropenia that occurs in newborns during states of increased demand (such as overwhelming bacterial sepsis) may, in part, be secondary to deficient CSF expression and production (Fig 1). Decreased CSF expression in the newborn may be secondary to a number of mechanisms including, but not limited to, instability of CSF mRNA, alteration in mRNA binding proteins, or immaturity of T-cell subsets (eg, naïve memory cells). Future studies elucidating the mechanisms associated with CSF mRNA instability will require the identification of cis and trans factors involved posttranscriptionally. Future studies may require a transgenic model of CSF deficiency to determine the interrelationship of CSF dysregulation and the development of neonatal neutropenia during overwhelming sepsis. Recent animal and both in vitro and in vivo human data suggest that exogenous administration of rhG-CSF results in enhancement of neonatal host defense and a reduction in the mortality rate during experimental overwhelming bacterial infection. Future phase II/III randomized double-blind multicenter trials are necessary to determine whether exogenous administration of CSFs can overcome the profound immaturity in neonatal host defense and result in an improvement in survival during overwhelming bacterial infection in the newborn.

ACKNOWLEDGMENT

The author thanks Linda Rahl for her excellent editorial assistance and the members of the CHOC Cancer Research Laboratory staff for their critique of this editorial.

REFERENCES

Therapeutic implications of dysregulated colony-stimulating factor expression in neonates [editorial; comment]

MS Cairo