# Granulocyte and Granulocyte-Macrophage Colony-Stimulating Factors in Cord and Maternal Serum at Delivery

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ABSTRACT. Impaired neutrophil responses contribute to the neonate's increased susceptibility to infection. Because granulocyte colony-stimulating factor (G-CSF) and granulocyte-macrophage colony-stimulating factor (GM-CSF) enhance granulocyte and macrophage number and function, their use in the management of neonatal sepsis may be beneficial. Little is known about the endogenous levels of G-CSF and GM-CSF. In adults, raised values for G-CSF, but not GM-CSF, have been demonstrated in patients with infection, and conflicting data has emerged regarding CSF levels in neonates. We have used an ELISA to measure maternal and cord serum G-CSF and GM-CSF at the time of delivery, with gestational age between 25 and 42 wk. In mothers, an inverse linear relationship between gestational age and GM-CSF levels ($p = 0.049$) was found, but no association with G-CSF levels was observed. In neonates, a quadratic association was found between GM-CSF levels and gestational age ($p = 0.019$), whereas G-CSF levels showed an inverse linear association ($p = 0.015$). In addition, an association was found between maternal and cord GM-CSF ($p = 0.007$) but not G-CSF levels in paired samples. The effect of gestational age on the cytokine levels could not be explained by the white cell count, the absolute neutrophil count, pregnancy-induced hypertension, or the presence of infection. We suggest that *1*) GM-CSF levels in mothers vary throughout gestation and may have a role in the maintenance of normal pregnancy; *2*) G-CSF and GM-CSF levels in neonates vary with gestational age and may have a developmental role; and *3*) G-CSF has theoretical benefit in the management of neonatal neutropenia and sepsis; clinical trials are now needed to establish its optimal use. (*Pediatr Res* 35: 164–168, 1994)

### Abbreviations

CSF, colony-stimulating factor
G-CSF, granulocyte colony-stimulating factor
GM-CSF, granulocyte-macrophage colony-stimulating factor
PIH, pregnancy-induced hypertension
WCC, white cell count
ANC, absolute neutrophil count

Neonatal sepsis remains an important clinical problem contributing to significant morbidity and mortality (1–5). The neonate is at increased risk of sepsis as a result of a number of immunologic defects (6), including impaired neutrophil function (7). The ability to enhance the neutrophil's response to infection would thus be an attractive adjunct to the conventional management of neonatal sepsis.

The hemopoietic growth factors G-CSF and GM-CSF, in addition to their role in the development of granulocyte and macrophage lineages, are capable of affecting mature cell function (8, 9). These effects include the "priming" of the neutrophil's response to inducers of the respiratory burst mechanism (10, 11), influencing chemotaxis (12–14) and phagocytosis (15, 16), and altering surface membrane receptor expression (17–20).

The clinical use of G-CSF and GM-CSF has included the acceleration of neutrophil recovery after chemotherapy and bone marrow transplantation, the treatment of neutropenia associated with infections, myelodysplasia or irradiation exposure, and congenital neutropenia (21–26). Their use in the management of infection has not been evaluated. Animal studies have suggested that an improved outcome in neonatal sepsis may be achieved using a combination of G-CSF or GM-CSF with antibiotics (27–29). Roberts *et al.* (30) have reported the use of G-CSF in an infected human neonate with neutropenia without adverse effect, resulting in an improvement in neutrophil count and elimination of sepsis.

Despite this increasing clinical use, little is known about the endogenous levels and significance of circulating G-CSF and GM-CSF. Serum GM-CSF levels are undetectable in normal healthy adults (9, 31), and raised levels have not been documented yet. In contrast, raised serum G-CSF levels have been found in 10% of normal adults (32), in the presence of infection (33, 34), and some cases of congenital neutropenia (35). In full-term healthy neonates, Laver *et al.* (36) measured G-CSF and GM-CSF using a bioassay and demonstrated raised levels of both cytokines when compared with normal adults. However, Cairo *et al.* (37, 38) were unable to detect G-CSF or GM-CSF in a similar group using an immunoassay. Data concerning serum G-CSF and GM-CSF levels in premature neonates have not been reported.

The aim of this study was to examine the endogenous levels of G-CSF and GM-CSF in cord blood at various stages of gestation and to examine the influence of maternal CSF levels, infection, and the reasons for premature delivery on the values obtained.

Ethical approval for the study was obtained from The Research Ethical Committee of the Faculty of Medicine of The Queen's University of Belfast.

## MATERIALS AND METHODS

*Subjects.* Mothers and babies were chosen at random from patients attending the Royal Maternity Hospital, Belfast. The

neonates were all singletons and ranged in gestational age from 25 to 42 wk at the time of delivery. Premature delivery was precipitated by the following maternal antenatal complications: PIH, diabetes mellitus, bleeding, or premature rupture of membranes (at <36 completed wk), or fetal distress. Thirty-three neonates were delivered vaginally, and 48 were delivered by cesarean section, of which 36 were elective procedures.

The incidence of infection in both mothers and babies was obtained from case note review. Maternal infection was diagnosed when pyrexia of 38°C or more occurred or when cultures of urine, sputum, or vaginal swab were found to be positive. Only episodes occurring 48 h before or 48 h after delivery were considered to have a potential influence on cytokine levels at the time of delivery. Neonatal infection was diagnosed by clinical assessment, with or without positive cultures of blood, urine, gastric aspirate, or bronchial secretions. Only early infection (39), occurring within the first 48 h of delivery, was considered to have a potential influence on cytokine levels at birth. The perinatal details of the study cases are summarized in Table 1.

*Serum samples.* After patients provided informed consent, blood samples were obtained from the mother and the umbilical cord at the time of delivery. The clotted samples were immediately separated, and the sera were stored in aliquots at $-20°C$ until analysis. This form of storage has previously been shown not to alter immunoassay results (40). At the same venipuncture, a sample was drawn to record the WCC and differential WCC.

*ELISA.* Commercially available assay kits for the measurement of G-CSF (Amgen Biologicals, Thousand Oaks, CA, sensitivity 5 ng/L) and GM-CSF (Medical Resources Ltd., Sydney, Australia, sensitivity 0.5 ng/L) were used. Both assays are based on the double-antibody sandwich method. Antibody precoated plates were incubated with standards or serum samples in duplicate. Rabbit anti-CSF, horseradish peroxidase–conjugated anti-rabbit antibody, and horseradish peroxidase substrate were added sequentially, with a washing step before each addition to remove uncaptured components. The final horseradish peroxidase substrate reaction was terminated by the addition of a weak acid solution before reading the absorbance on an ELISA plate reader (Dynatech MR 700, Dynatech, Cambridge, MA). No cross-reactivity with other cytokines or other serum components has been reported (manufacturer's data). A standard curve constructed with the means of the duplicate results was used to determine the values for CSF in the test sera.

*Statistical analysis.* Mother-baby pairs were divided into five groups by gestational age. Because of the occurrence of values below the sensitivity of the assays, mean and SD could not be calculated for each group. Hence the actual values obtained in the ELISA were catagorized into "raised" and "not raised"

groups, with reference to the expected normal adult value. The normal adult values were less than 30 ng/L for GM-CSF (9) and less than 50 ng/L for G-CSF (8). The values for WCC and ANC were transformed using natural logarithms before analysis. The continuous variables, namely, gestation, WCC, and ANC, were evaluated for association with CSF levels using independent samples $t$ tests. The analysis of WCC and ANC were then adjusted for the confounding effects of gestation using logistic regression analysis. The variables PIH and infection were classified as either present or absent and were initially evaluated for association with CSF levels using the $x^2$ test. These associations were then reexamined in five strata defined by gestational age, using the Mantel-Haenstal stratified $x^2$ test to adjust for the confounding effects of gestation.

## RESULTS

The ranges of results obtained by ELISA for each gestational age group are shown in Figures 1 through 4. Cases in which the levels of cytokine were below the sensitivity of the assay were, for illustrative purposes only, assigned a value of 5 ng/L (GM-CSF) and 20 ng/L (G-CSF). Tables 2 and 3 give a summary of the statistical analysis.

GM-CSF values were obtained in 56 maternal sera, of which 18 had raised values ranging from 48 to 190 ng/L (Fig. 1). An inverse linear association between GM-CSF level and stage of gestation was found ($p = 0.049$).

GM-CSF values were obtained in 58 cord sera, of which 14 had raised values ranging from 32 to 200 ng/L (Fig. 2). A quadratic association between gestational age and cord GM-CSF level was demonstrated ($p = 0.019$), with the greatest frequency of raised values occurring between 28 and 36 wk. In addition, an association between GM-CSF levels in maternal and cord sera was noted ($p = 0.007$).

G-CSF values were obtained in 46 maternal sera, of which six were found to have raised levels ranging from 250 to 1000 ng/L (Fig. 3). These levels occurred between 28 and 36 wk, but no association with gestational age could be demonstrated.

G-CSF values were obtained in 64 cord sera, of which 15 were found to have raised values ranging from 75 to 3750 ng/L (Fig. 4). An inverse linear association between G-CSF level and gestational age was shown ($p = 0.015$). No correlation was seen between maternal and cord G-CSF levels.

G-CSF or GM-CSF levels in cord or maternal sera were not

Table 1. *Summary of perinatal characteristics*

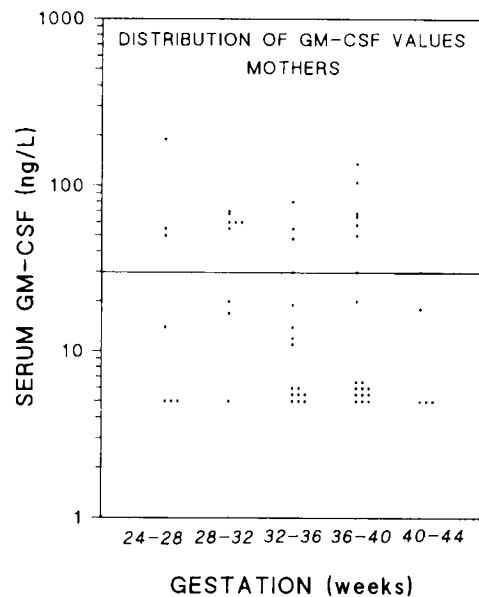| | Group | | | | |
|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 |
| Gestation (wk) | 24–28 | 28–32 | 32–36 | 36–40 | 40–44 |
| Total number of mother-baby pairs | 9 | 17 | 23 | 28 | 5 |
| Delivery | | | | | |
| Normal | 3 | 6 | 13 | 8 | 3 |
| Cesarean section | 6 | 11 | 10 | 19 | 2 |
| Elective | 2 | 7 | 8 | 17 | 0 |
| Perinatal complications | | | | | |
| PIH | 4 | 8 | 7 | 2 | 0 |
| Diabetes | 0 | 1 | 2 | 0 | 0 |
| Premature R.O.M.* | 5 | 5 | 10 | 0 | 0 |
| Prolonged R.O.M. | 0 | 0 | 2 | 0 | 0 |
| Maternal infection | 2 | 4 | 4 | 3 | 1 |
| Neonatal infection | 2 | 2 | 1 | 0 | 0 |

* R.O.M., rupture of membranes.



Fig. 1. Distribution of maternal serum GM-CSF values at delivery between 24- and 42-wk gestation.
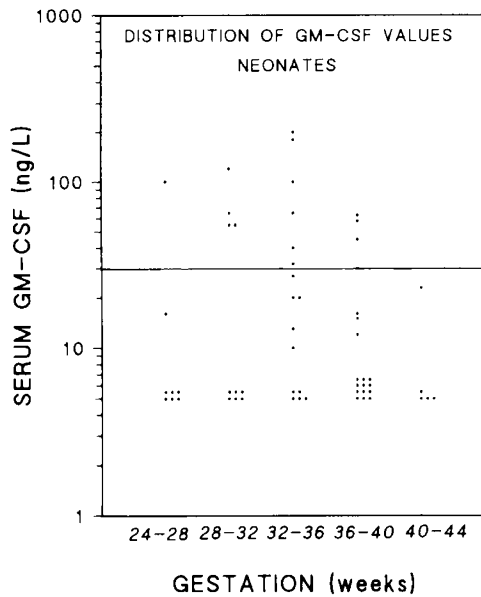
Fig. 2. Distribution of cord serum GM-CSF values at delivery between 24- and 42-wk gestation.



Fig. 3. Distribution of maternal serum G-CSF values at delivery between 24- and 42-wk gestation.



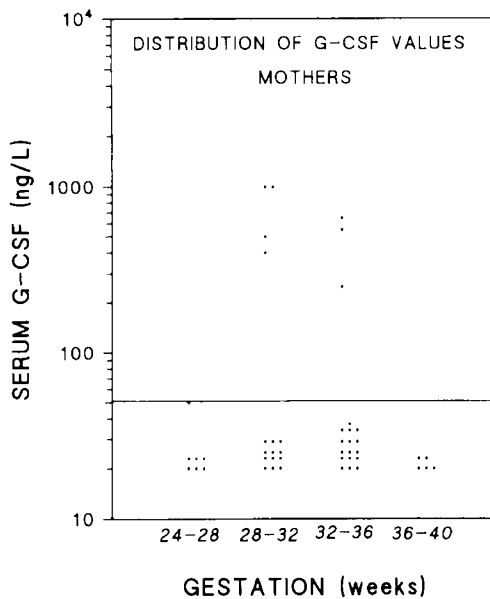Fig. 4. Distribution of cord serum G-CSF values at delivery between 24- and 42-wk gestation.

associated with the WCC or ANC or with the presence of infection or PIH. In addition, the observed effect of gestation could not be accounted for by any of these variables (Tables 2 and 3).

## DISCUSSION

The role of G-CSF and GM-CSF in the control of hematopoiesis and their effects on mature cell function have been well established (21–26). Measurement of circulating CSF in adults has indicated that under normal conditions both G-CSF and GM-CSF are either undetectable or present in very small amounts (31, 32). The levels during pregnancy have not been previously described.

Our data suggest that although maternal G-CSF levels do not vary throughout gestation, a small but significant change occurs in maternal GM-CSF levels. GM-CSF is detectable in increased amounts fr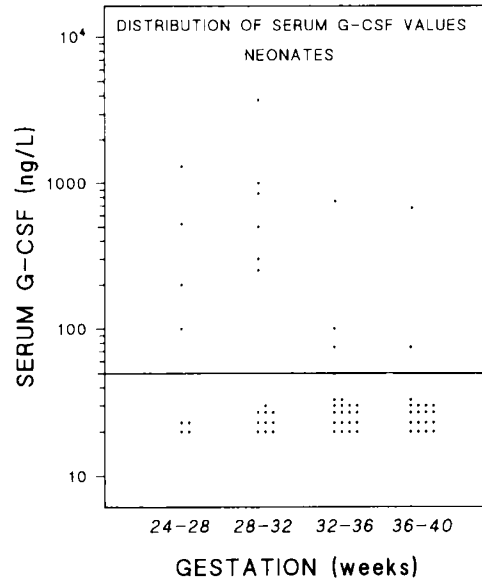om at least 25 wk, but normal nonpregnant adult levels are apparent at full term. The role of GM-CSF during human pregnancy remains obscure, but previous animal studies have demonstrated its production in uterine luminal or epithelial cells during early and midpregnancy (41, 42). The association between GM-CSF levels in 56 pairs of maternal and cord samples would be in keeping with a placental source of GM-CSF and alludes to a role in the growth and development of the fetoplacental unit. The lack of association in the corresponding G-CSF levels suggests an alternative influence, although placental production of G-CSF has also been demonstrated (43). The presence of receptors for G-CSF and GM-CSF on placental tissue (44, 45) would further support a role for these cytokines in normal pregnancy.

Raised serum levels of G-CSF have been demonstrated in acute infections (33, 34). The lack of association between infection, documented in 20% of mothers in the study, and G-CSF levels may reflect an altered response to infection during pregnancy.

In neonates, difficulty in the accurate assessment of sepsis, particularly in the premature infant (46), may have led to the underrepresentation of "infection" in this group and masked an association with a raised CSF. However, in our group of premature neonates, five (6%) had documented early infection, a figure in keeping with the suggested rate of infection in premature infants (4). Furthermore, 31% of neonates had raised levels of G-CSF, and 32% had raised levels of GM-CSF, suggesting that infection probably does not account for all of the patients with raised CSF levels.

Neonatal G-CSF and GM-CSF levels were not associated with the presence of PIH or with the WCC or ANC, suggesting that variation in endogenous levels of G-CSF and GM-CSF with progression of gestation is independent of these variables. Our findings are in keeping with those of Cairo *et al.* (37, 38), who were unable to demonstrate G-CSF or GM-CSF in cord sera of full-term neonates using an ELISA, but our results are at variance with those of Laver *et al.* (36), who found high levels of both G-CSF and GM-CSF in full-term cord sera using a bioassay. This discrepancy may be accounted for by the different assay techniques used. A bioassay may be influenced by other stimulatory or inhibitory factors in test sera (31), whereas ELISA is specific for a single factor (31, 47), although it provides no functional information. We would then suggest that raised G-CSF and GM-CSF levels found in cord blood from premature neonates may have a role in fetal growth and development.

These observations are also of interest when considering the

Table 2. *Mothers: linear associations*

| Variable | GM-CSF | | | p value adjusted for gestational age | G-CSF | | | p value adjusted for gestational age |
|---|---|---|---|---|---|---|---|---|
| | >30 ng/L (n = 18) | <30 ng/L (n = 38) | p value | | >50 ng/L (n = 6) | <50 ng/L (n = 40) | p value | |
| Gestation mean (SD)* | 232 (32) | 250 (32) | 0.049 | | 223 (16) | 228 (26) | 0.64 | |
| WCC | | | | | | | | |
| Median | 13.2 | 15.4 | 0.60 | 0.33 | 18.4 | 13.0 | 0.62 | 0.68 |
| Quartiles | 11.4, 17.4 | 10.9, 22.4 | | | 12.5, 27.7 | 10.6, 19.0 | | |
| ANC | | | | | | | | |
| Median | 11.1 | 14.8 | 0.65 | 0.46 | 13.4 | 11.0 | 0.53 | 0.48 |
| Quartiles | 8.8, 25.3 | 8.7, 49.1 | | | 9.3, 25.3 | 7.9, 17.9 | | |
| PIH | 4/18 (22%) | 10/38 (26%) | 0.51 | 0.74 | 1/6 (17%) | 17/40 (43%) | 0.16 | 0.18 |
| Infection | 2/18 (11%) | 6/38 (13%) | 0.59 | 0.87 | 1/6 (17%) | 8/40 (20%) | 0.60 | 0.90 |

* Gestation period in days.

Table 3. *Cord blood: linear associations*

| Variable | GM-CSF | | | p value adjusted for gestational age | G-CSF | | | p value adjusted for gestational age |
|---|---|---|---|---|---|---|---|---|
| | >30 ng/L (n = 14) | <30 ng/L (n = 44) | p value | | >50 ng/L (n = 15) | <50 ng/L (n = 50) | p value | |
| Gestation mean (SD)* | 237 (24) | 245 (35) | 0.43 0.019† | | 221 (28) | 239 (25) | 0.02 | |
| WCC | | | | | | | | |
| Median | 9.0 | 11.5 | 0.43 | 0.49 | 9.1 | 11.6 | 0.43 | 0.67 |
| Quartiles | 7.1, 18.2 | 8.6, 14.7 | | 0.5 | 8.4, 12.6 | 8.1, 14.3 | | |
| ANC | | | | | | | | |
| Median | 3.6 | 4.4 | 0.53 | 0.29 | 3.5 | 4.1 | 0.97 | 0.20 |
| Quartiles | 2.8, 6.3 | 2.8, 6.3 | | 0.3 | 3.1, 4.3 | 2.6, 6.1 | | |
| PIH | 4/14 (29%) | 10/44 (23%) | 0.38 | 0.73 | 3/15 (20%) | 18/50 (36%) | 0.20 | 0.078 |
| Infection | 1/14 (7%) | 2/44 (5%) | 0.55 | 0.61 | 1/15 (7%) | 3/50 (6%) | 0.67 | 0.95 |
| Maternal GM-CSF raised | 8/13 (62%) | 10/43 (23%) | 0.005 | 0.007 | 3/14 (21%) | 4/33 (9%) | 0.37 | 0.67 |

* Gestation period in days.
† p value for quadratic association.

potential use of exogenous G-CSF or GM-CSF in the management of neonatal sepsis. Experience with the use of these factors in children and adults has indicated that G-CSF is well tolerated, is associated with few side effects, and can produce an improvement in neutrophil counts and a reduction in the number and severity of infections (8, 48–52). In contrast, GM-CSF has been associated with more severe side effects, particularly at higher dosage, and although neutrophil numbers were improved, the number of infective episodes was not significantly altered (22, 26). This difference in efficacy may relate to the respective effects of G-CSF and GM-CSF on neutrophil function. GM-CSF inhibits chemotaxis at high dosage (12, 13) and up-regulates neutrophil adhesion molecules (17–19), leading to sequestration of neutrophils in the circulation (53) and promotion of localized vascular damage (54). GM-CSF also promotes the release of secondary mediators such as tumor necrosis factor and IL-1 (55), which also may contribute to the production of side effects. Recently, however, Cebon et al. (56) demonstrated that the toxic effects of GM-CSF could be dissociated from its proliferative and priming effects by using s.c. rather than i.v. administration. In comparison, the effects of G-CSF are confined to the neutrophil and are not as potent in promoting neutrophil adhesion, chemotaxis, and respiratory burst activity (57).

These observations, in combination with our findings of higher levels of G-CSF and GM-CSF in the premature neonate, would suggest that G-CSF may be less toxic and more effective as an adjuvant to standard treatment of neonatal sepsis, particularly in the setting of neotropenia. GM-CSF could have a theoretical role in the management of fungal infection when the enhancement of macrophage function could be beneficial. Clinical trials of these agents are now required to establish criteria for their use and optimum therapeutic dosage.

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