Serum Concentrations of Granulocyte Colony-Stimulating Factor in Healthy Term and Preterm Neonates and in Those With Various Diseases Including Bacterial Infections

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The neonate is uniquely susceptible to severe and overwhelming bacterial infections. One of the most important deficits in the neonatal host defense system seems to be a quantitative and qualitative deficiency of the myeloid and phagocytic system. Future optimal therapy of neonatal sepsis may include the use of adjuvant immunologic therapy. Granulocyte colony-stimulating factor (G-CSF) has been shown to induce neutrophilia and to enhance mature effector neutrophil function. To evaluate the role of G-CSF with respect to infection, we examined serum levels of G-CSF in term and preterm neonates, using an enzyme-linked immunosorbent assay method. G-CSF levels in healthy neonates showed peak levels up to 7 hours after birth, followed by an increase in total neutrophil cell (TNC) counts. Both G-CSF levels determined between 4 and 7 hours after birth and peak TNC counts correlated with the gestational age of the neonates. The state of nutrition, maternal treatment with glucocorticoids, maternal infection and hypotension, and the mode of delivery influenced peak G-CSF levels. Neonates with signs of infection between 4 and 7 hours after birth had higher levels of G-CSF than did healthy neonates (1.312 ± 396 pg/mL v 176 ± 19 pg/mL). In conclusion, the presented results of serum concentrations of G-CSF in relation to TNC counts and various diseases suggest an important role of G-CSF in the regulation of granulopoiesis during the neonatal period.

MATERIALS AND METHODS

Patients

The studied population included infants admitted to the Department of Pediatrics, University Hospital of Mannheim, during the 6 months from July 1, 1992 through January 1, 1993 (n = 156). Neonates older than 12 hours at the time of admission and infants with severe life-threatening malformations were excluded. Informed written consent was obtained from the parents of each newborn.

Bacterial infection in an ill neonate was confirmed in case of a positive blood culture. Infection was presumed in a clinically symptomatic child in case of Pneumoniasis intestinalis or pneumonia on chest roentgenogram and whenever two or more of the following criteria were fulfilled: positive C-reactive protein, fibrinogen less than 100 mg/dL or greater than 400 mg/dL, platelet count less than 150 × 10^9/L, immature to total neutrophil ratio ≥0.16, positive skin and gastric culture, maternal infection (fever, signs of infection, or rupture of membrane ≥24 hours), nasopharyngeal and urinary viral cultures and stool rotavirus tests were performed when clinically indicated.

Antibiotic treatment was performed as thought to be clinically indicated. Cefotaxime and amoxicillin were the first line regime for children weighing less than 1,500 g, amoxicillin and gentamicin for children greater than 1,500 g, which was later on changed according to the results of bacterial culture or clinical course.

The diagnosis of maternal pregnancy-induced hypertension was based on two measurements obtained at least 6 hours apart of a
diastolic blood pressure greater than 90 mm Hg or a systolic blood pressure greater than 140 mm Hg, or an elevation from base line of greater than 30 mm Hg in the systolic pressure or of greater than 15 mm Hg in the diastolic pressure.30

Blood Cell Counts

Routinely, a complete blood cell (CBC) count was obtained from all infants on admission and subsequently as thought to be indicated by the clinicians responsible for the infant's care. The CBC included a nucleated cell count (Sysmex F 800 microcellcounter), platelet count, and a 100-cell differential count. The nucleated cell count represented the total white blood cell (WBC) count, corrected for the presence of nucleated red blood cells. A total neutrophil cell (TNC) count was calculated for each CBC count by multiplying the total WBC by the percentage of polymorphonuclear leukocytes and band forms. An I/T neutrophil ratio was calculated for each CBC count as the combined percentiles of immature neutrophils (band forms, metamyelocytes, and myelocytes) divided by the total percentage of neutrophils in the peripheral blood. Blood samples for CBC count were obtained from heelpricks and peripheral venipunctures.

Enzyme-Linked Immunosorbent Assay (ELISA) for Detecting Human G-CSF

Together with clinically indicated blood tests, an additional amount of 0.6 mL blood was taken for G-CSF determination by peripheral venipuncture. Samples were obtained usually upon admission, between days 4 and 6 of life, between weeks 2 and 3 of life, and whenever an infection was suspected. In some cases, cord blood was also available. The blood specimens were immediately centrifuged and the serum was stored at -36.4°F. For determination of G-CSF in human serum, the sandwich enzyme immunoassay technology was used. All incubation steps were performed in irradiated microtiter plates from polystyrol with 96 F-wells (Maxisorp; Nunc) at room temperature with agitation on a shaker table. Microtiter plates, precoated with streptavidine, were coated with 125 μL/well of biotinylated mouse monoclonal anti-G-CSF antibody (clone KM 341; Kyowa) in a concentration of 2 µg/mL and incubated for 1 hour. The plate was then washed three times with 0.9% (wt/vol) NaCl/0.1% (wt/wt) Tween 20. Serum samples or standards were diluted with an equal volume of incubation buffer (Enzymun LH-standards were measured in duplicate and the absorbance means were calculated. From the standard means, a calibration curve was derived according to the 4 parameter logistic model (Rodbard equation) and used for conversion of the samples' absorbances into concentrations. Using this method, the lower limit of detection was 15 pg G-CSF/mL.

Statistical Analysis

Statistical analysis was performed using the Mann-Whitney test (for unpaired continuous variables, two-tailed P value) and the χ² test with Yates (continuity) correction for categorical variables. For multiple comparisons, analysis of variance (ANOVA) with Bonferroni correction. For calculation of correlation coefficient r, least squares linear regression was performed. Results are expressed as rate percent and mean ± standard error (SE) unless otherwise expressed.

RESULTS

Serum Concentrations of G-CSF and TNC Counts of Different Newborns Without Signs of Infection at Birth, at Various Times of Admission, and Later on Until Week 3 of Life

Mean serum concentrations of G-CSF at birth were higher in neonates older than 37 gestational weeks compared with those in neonates with gestational age 32 to 37 weeks. Cord blood (CB) values of these neonates were 261 ± 23 pg/mL versus 126 ± 68 pg/mL (P = .048) (Fig 1). No difference was detectable in CB G-CSF values of neonates with gestational age 32 to 37 weeks compared with those with gestational age 26 to 31 weeks (141 ± 67 pg/mL).

At admission, mean G-CSF levels of neonates with gestational age 32 to 37 weeks and of those older than 37 weeks were highest between 4 and 7 hours of life, with peak levels of 158 ± 22 pg/mL versus 228 ± 40 pg/mL, respectively. Neonates with gestational age 26 to 31 weeks had a mean G-CSF value of 77 ± 30 pg/mL between 4 and 7 hours of life (difference between gestational age groups P = .07). In neonates younger than 32 gestational weeks, no data can be shown for the time intervals between 1 through 3 and the 3 through 12 hours of life, because no clinically indicated peripheral venipuncture was performed. The relationship of
G-CSF values between 4 and 7 hours of life to gestational age is shown in Fig 2 (n = 64; correlation coefficient r = .48; P = .0002; n = 64 data points). G-CSF values between 4 and 7 days of life and 2 and 3 weeks of life were lower compared with the time interval between 4 and 7 hours of life and there was no significant difference between the three gestational age groups.

Mean TNC counts (Fig 3) were highest at the time interval between 7 and 12 hours of life in all three gestational age groups. The difference of mean TNC counts between the gestational age groups was significant until 72 hours of life (P < .005), except for direct comparison of neonates younger than 32 gestational weeks of age to neonates 32 to 37 gestational weeks of age (significant difference of these two gestational age groups only in neonates younger than 6 hours of life, Bonferroni P value < .001).

**G-CSF Values and TNC Counts in Twins**

At the time of admission, G-CSF values of twins (dizygotic, n = 9) were lower in the first twin compared with the second born (117 ± 23 pg/mL [n = 9] v 252 ± 71 pg/mL [n = 9]; P = .02). In twins delivered spontaneously (n = 5), the respective values were 122 ± 39 pg/mL versus 307 ± 125 pg/mL; in twins delivered by caesarian section (n = 4), the respective values were 110 ± 25 pg/mL versus 184 ± 40 pg/mL.

After that time, G-CSF values of the second born determined between days 4 and 7 and weeks 2 and 3 of life were slightly higher compared with those of the first born (44 ± 17 v 26 ± 7 pg/mL and 31 ± 17 v 16 ± 4 pg/mL). However, this difference was not statistically significant. The difference in TNC counts of twins at any time until week 3 of life was not significant.

**G-CSF Values, TNC Counts, and Rate of Infections in Newborns Appropriate and Small for Gestational Age**

Appropriate for gestational age (AGA) newborns with birth weight ≥2,500 g and gestational age ≥38 weeks without signs of infection (n = 28) had different G-CSF levels and TNC counts compared with those small for gestational age (SGA) newborns (birth weight <2,500 g, gestational age ≥38 weeks) (n = 13). G-CSF levels determined between 4 and 7 hours of life were higher in AGA neonates (284 ± 54 pg/mL, n = 13) compared with SGA neonates (114 ± 31 pg/mL, n = 8) (P = .05). Later on, there were no significant differences in G-CSF levels between the two groups: G-CSF values between days 4 and 7 of life, 24 ± 6 pg/mL (n = 13) v 21 ± 6 pg/mL (n = 12), and between 2 and 3 weeks of life, 24 ± 14 pg/mL (n = 7) v 8 ± 4 pg/mL (n = 9). Mean TNC counts determined between 4 and 7 hours and between days 4 and 7 of life were higher in AGA neonates (12.7 ± 1.4 v 9.2 ± 1.7 × 10⁹/L and 4.2 ± 0.5 v 2.7 ± 0.2 × 10⁹/L; P = .04). Of a total number of 34 AGA neonates, 6 infants showed signs of infection compared with 3 of 16 SGA neonates.

**Effect of Perinatal Events on G-CSF Levels, TNC Counts, and Rate of Infections**

**Maternal hypertension.** Neonates born to hypertensive mothers (n = 21) compared with neonates born to non-hypertensive mothers without signs of infection (n = 100) tended to have lower G-CSF levels (146 ± 42 pg/mL [n = 10] v 182 ± 21 pg/mL [n = 54]) and TNC counts (7.4 ± 0.8 v 10.0 ± 0.6 × 10⁹/L) between 4 and 7 hours of life (not significant). Later on, G-CSF levels and TNC counts showed no difference. The rate of presumed infections occurring until day 5 of life was equal in both groups.

**Maternal treatment with glucocorticoids for prevention of respiratory distress syndrome (RDS).** Neonates without signs of infection born to mothers treated with glucocorticoids (n = 26) tended to have lower G-CSF levels between 4 and 7 hours of life than those without maternal glucocorticoid treatment (n = 40, comparable gestational age) (117 ± 18 pg/mL [n = 19] v 174 ± 41 pg/mL [n = 18]; not significant). In contrast, TNC counts at that time were higher in...
neonates of corticoid-treated mothers \( (8.6 \pm 0.9 \times 6.8 \pm 0.6 \times 10^9/L; P = .05) \). Later on, G-CSF levels and TNC counts showed no difference, which was also true for the rate of presumed infections in both groups.

**RDS.** There was no difference in G-CSF levels of noninfected neonates with \( (n = 7) \) and without RDS \( (n = 60, \) comparable gestational age \( ) \) at any time, nor for TNC counts between 4 and 7 hours of life. However, between days 4 and 7 and weeks 2 and 3 of life, TNC counts were higher in neonates with RDS \( (7.6 \pm 1.0 \times 3.2 \pm 0.2 \times 10^9/L; P = .001; \) and \( 4.5 \pm 0.1 \times 3.5 \pm 0.5 \times 10^9/L; P = .02) \). The rate of presumed infections was highest in newborns with RDS. Of a total of 12 infants with RDS, 5 had signs of infection (positive blood culture, \( n = 3 \) ) until the day 5 of life, compared with 8 of 68 infants without RDS (positive blood culture, \( n = 1 \) ) \( (P = .02) \).

Perinatal asphyxia defined as a 1-minute APGAR score \( \leq 5 \) had no influence upon G-CSF levels, TNC counts, and rate of presumed infections.

**Maternal infection.** Neonates born to mothers with signs of bacterial infections (positive blood culture, fever \( >38.5^\circ C, \) CRP \( >120 \mu g/mL, \) premature rupture of membranes, and choioriamnionitis) \( (n = 17) \) but without signs and symptoms of neonatal infection \( (n = 12) \) had higher levels of G-CSF determined between 4 and 7 hours of life compared with neonates of noninfectious mothers \( (709 \pm 374 \mu g/mL; n = 5 \times 176 \pm 19 \mu g/mL; n = 64; P = .05) \). Between days 4 and 7 and weeks 2 and 3 of life, there was no difference in G-CSF levels between the two groups \( (31 \pm 6 \times 29 \pm 4 \mu g/mL; 18 \pm 8 \times 25 \pm 5 \mu g/mL) \). Mean TNC count of neonates of infectious mothers between 4 and 7 hours was \( 11.6 \pm 2.3 \times 10^9/L \) compared with \( 9.1 \pm 0.6 \times 10^9/L \) of noninfectious mothers (not significant). Later on, TNC counts in both groups were similar. The rate of presumed infections of neonates born of infectious mothers was \( 29\% \) compared with \( 14\% \) of noninfectious mothers \( (P = .09) \).

**Neonatal Infection: Incidence, G-CSF Values, and TNC Counts**

The overall rate of presumed bacterial infections was \( 22\% \) \( (n = 35) \). Blood culture was positive in \( 26\% \) of cases of neonatal infection. Positive skin and gastric cultures were found in \( 29\% \) and \( 34\% \), respectively \( (B \) streptococcus, \( n = 6; \) Staphylococcus epidermidis, \( n = 6; \) Escherichia coli, \( n = 3; \) Pseudomonas aeruginosa, \( n = 2; \) Enterococci, \( n = 2; \) others, \( n = 3) \). There were 2 cases of NEC \( (6\% \) \), 1 case of pneumonia, and no case of proven viral infection.

The overall rate of infections correlated with the gestational age of the neonates, occurring more often in younger infants. Of 17 neonates younger than 32 gestational weeks, 10 had signs of infection during their hospital stay \( (59\%) \). Of neonates with gestational age 32 to 37 weeks \( (n = 85) \), 15 had signs of infection \( (18\%) \). Of neonates older than 37 gestational weeks of age \( (n = 54) \), 10 had signs of infection \( (19\%) \) \( (P = .0007) \). However, younger infants stayed in the hospital for a longer period of time, thus having a greater chance of acquiring a nosocomial infection. Therefore, it is necessary to analyze the rate of infections during the first 5 days of life. Again, there was a relation of the rate of infections to gestational age: \( 29\% \) \( (n = 5) \) in neonates less than 32 gestational weeks of age, \( 12\% \) \( (n = 10) \) in neonates 32 to 37 gestational weeks of age, and \( 19\% \) \( (n = 8) \) in neonates older than 37 gestational weeks of age \( (P = .03) \).

With respect to the dynamics of G-CSF levels and TNC counts during the first 5 days of life \( (Figs 1 \text{ and } 3) \), neonates with signs of infection between 4 and 7 hours of life were compared with neonates without signs of infection at that time \( (Fig 4) \). G-CSF levels of infected neonates at that time \( (n = 12) \) were \( 1,312 \pm 396 \mu g/mL \) compared with \( 176 \pm 19 \mu g/mL \) in noninfected neonates \( (n = 64) \) \( (P < .0001) \). TNC count at that time was \( 11.9 \pm 3.9 \times 9.2 \pm 0.6 \times 10^9/L \) (infected \( v \) noninfected; \( P = \) NS), with an immature neutrophil cell count of \( 1.9 \pm 0.8 \times 10^9/L \) \( (T/I \) ratio \( = 0.16 \pm 0.06) \). Mean TNC counts as well as the number of immature neutrophil cells were highest between 7 and 12 hours of life.

G-CSF levels determined between days 4 and 7 of life were not significantly different in infected versus noninfected neonates \( (47 \pm 18 \times 30 \pm 4 \mu g/mL) \). However, TNC counts of infected neonates differed significantly: \( 7.8 \pm 2.1 \times 3.5 \pm 0.2 \times 10^9/L; P = .0047 \).

Between weeks 2 and 3 of life, G-CSF levels and TNC counts of infected and noninfected neonates were equal. G-CSF levels of neonates determined at the time of first clinical signs of neonatal infection during the first 5 days of life and after that time were not significantly different compared with 4 and 7 hours of life, with a minimum of \( 216 \mu g/mL \) and a maximum of \( 350,000 \mu g/mL \).

**DISCUSSION**

Serum concentrations of G-CSF in term and preterm neonates without signs of infection reached peak levels during the first 7 hours of life. However, mean TNC counts were highest between 7 and 12 hours of life, suggesting a time-
related delay of the effect of G-CSF on peripheral neutrophil cell counts. The observed priming time of endogenous G-CSF levels on neutrophil kinetics in human neonates is comparable to studies of exogenous applied G-CSF in animals reporting priming times of 6 and 24 hours, respectively, and in humans with various disorders of granulopoiesis.

G-CSF levels determined between 4 and 7 hours after birth and peak TNC counts showed a correlation to the gestational age of the newborns. G-CSF values determined between days 4 and 7 of life and later on until week 3 of life were in the range of values of healthy adults.

Studies in dizygotic twins showed higher serum concentrations of G-CSF in the second born compared with the first born. This difference was more pronounced when taking into account the mode of delivery, showing a greater difference in twins delivered spontaneously compared with twins delivered by caesarian section.

Newborns of appropriate size for gestational age had higher values of G-CSF determined between 4 and 7 hours after birth, resulting in higher TNC counts compared with newborns small for gestational age. Our study failed to show a higher mortality rate and incidence of infections in SGA newborns, mostly because healthy AGA newborns were not admitted to the department of pediatrics. However, reports about the mortality risk of growth-retarded infants suggest an increased risk and incidence of neonatal infection compared with normally grown infants of similar gestational age.

There are conflicting reports regarding perinatal events like maternal hypertension and perinatal asphyxia and their effect on TNC counts. Neonates born to hypertensive mothers tended to have lower G-CSF values and TNC counts soon after birth (not significant); perinatal asphyxia also did not affect G-CSF values and TNC counts.

Neonates born to mothers treated with glucocorticoids for prevention of respiratory distress syndrome had slightly lower serum concentrations of G-CSF determined between 4 and 7 hours after birth. This may be in accordance to in vitro results indicating a suppressive effect of dexamethasone on the production of G-CSF in human endothelial cells supplemented with tumor necrosis factor-α.

In neonates born to mothers with signs of maternal infection, serum concentrations of G-CSF determined soon after birth were about 4 times higher compared with neonates of noninfectious mothers. These increased G-CSF levels resulted in a statistically nonsignificant increase in TNC counts.

Term and preterm newborns with signs of bacterial infection soon after birth had about sevenfold higher G-CSF values compared with noninfected neonates. Thus, G-CSF values in neonates with infections are in the same range like in adults with infections. Again, the increase in immature and TNC counts was time-delayed. The range of TNC counts was much broader compared with noninfected neonates, reflecting, in part, the possible occurrence of neutropenia along with neonatal infection in contrast to adults.

The relationship of G-CSF values and TNC counts to gestational age might reflect at least in part a reason for the increased susceptibility of neonates to bacterial infections. Further studies of G-CSF in infected neonates are needed to assess the role of this cytokine in life-threatening infections during the first days of life.

ACKNOWLEDGMENT

The authors thank Dagmar Knodel for technical assistance and the nurses of the neonatal care unit for their cooperation.

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