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GRANULOCYTE AND GRANULOCYTE – MACROPHAGE COLONY – STIMULATING FACTORS, THEIR RECEPTORS AND INTERLEUKIN – 3 LEVELS IN NEWBORNS*

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SUMMARY: Coşkun M, Kardelen F, Oygür N, Ündar L, Savaş A, Yeğin O. (Departments of Neonatology, Clinical Immunology and Hematology, Akdeniz University Faculty of Medicine, Antalya, Turkey). Granulocyte and granulocyte-macrophage colony-stimulating factors, their receptors and interleukin-3 levels in newborns. Turk J Pediatr 1997; 39: 295-301.

Serum levels of granulocyte-macrophage colony-stimulating factor (GM-CSF), granulocyte colony-stimulating factor (G-CSF) and interleukin-3 (IL-3) as well as neutrophil counts were studied on the first, second and fifth days after birth, in order to elucidate the relations between neutrophil kinetics and these hematopoietic factors. The G-CSF and GM-CSF receptors on neutrophils were also investigated in 16 healthy newborns.

G-CSF and GM-CSF receptor-positive neutrophil percentages were not different from those in the peripheral blood of adult controls. The receptor densities, assessed by mean fluorescence channel number, were also similar in newborn and adult neutrophils.

The mean serum concentrations of G-CSF were high (191 pg/ml) on the first day of life and gradually decreased on the second (128 pg/ml) and fifth (112 pg/ml) days. A statistically significant correlation ($p < 0.05$) was found between G-CSF levels and absolute neutrophil counts (ANC). Furthermore, the percentage of decrease in G-CSF levels correlated significantly with the percentage of decrease in ANC ($p < 0.001$). GM-CSF levels were also high, though less striking, on the first day of life (9.5 pg/ml), remained at high levels on the second (10 pg/ml), and gradually decreased on the fifth (8.8 pg/ml) day. IL-3 levels were high (110 pg/ml) on the first day of life and remained at high levels on the second (138 pg/ml) and fifth (138 pg/ml) days. We found that the IL-3, GM-CSF and G-CSF levels were elevated during the first week of postnatal life. Our findings suggest that significant changes in the levels of the growth factors are likely to be the cause of significant leukocyte blood picture changes during the first week of life. We found normal GM-CSF and G-CSF receptors in uninfected newborns. *Key words: newborn, G-CSF, GM-CSF, IL-3, neutrophil.*

Neutrophil production and function in the neonatal period are immature, and the newborn has a tendency to develop neutropenia during severe infections[1,2]. It is also well known that the leukocyte blood picture changes significantly during

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the early days of postnatal life[3-5]. The number and functional activity of neutrophils available for host defense, depends on their production, which is regulated by growth factors granulocyte-macrophage colony-stimulating factor (GM-CSF), granulocyte colony-stimulating factor (G-CSF) and interleukin-3 (IL-3). These hematopoietic growth factors exert their biologic activities through binding to their specific receptors. Little is known about the regulation of GM-CSF and G-CSF receptor expression and their status in the neonatal period[6-10].

The levels of G-CSF and GM-CSF receptors on neonatal neutrophils were measured with flow cytometry. The serum levels of GM-CSF, G-CSF and IL-3 and the neutrophil counts on the first, second and fifth days after birth were studied in order to elucidate the possible relations between neutrophil kinetics and these hematopoietic factors.

Material and Methods

Subjects: The peripheral blood of 25 healthy term neonates was studied on the first, second and fifth days after birth. GM-CSF and G-CSF receptors levels were studied in the cord blood of 16 healthy term neonates and the peripheral blood of 10 adult controls. Newborns with complicated deliveries or born to mothers with any sign of infection were not enrolled in the study. Newborns showing any signs of infection during the observation period were also excluded. The study was sanctioned by the Akdeniz University Ethics Committee. Peripheral blood samples were collected in sterile tubes and allowed to coagulate in the refrigerator (+4 °C) for 15-20 minutes; the tubes were then centrifuged at 600 g for 10 minutes, and samples from the serum were removed and kept frozen at -60 °C until they were assayed. White blood cell and differential counts were determined from each sample.

Cytokine Determinations: We used commercially available ELISA (Research and Diagnostics Inc, Minneapolis, MN, USA) kits specific to G-CSF, GM-CSF and IL-3 to assess the levels of these cytokines. All samples were tested in duplicate, and all sera were studied simultaneously.

GM-CSF and G-CSF Receptor Determination: Biotinylated GM-CSF, G-CSF and Avidin-FITC were purchased from British Bio-Technology Ltd, Oxford U.K. Quantitative fluorescence analyses were performed using flow cytometry (Coulter Epics-Profile II, Coulter Electronics, Inc., Hialeah, FL, USA). Then neutrophil population was identified using forward and riht-angle light scatter characteristics. Avidin-FITC was used as a negative control. The percentage of receptor-positive cells was defined as the percentage of gated events with a fluorescence intensity greater than the negative marker. Events within the acquisition gate were more than 95 percent positive for anti-CD13 binding. The mean fluorescence channel number was used as an arbitrary unit of receptor fluorescence intensity, i.e., the amount of receptor on the neutrophil surface.

The data were analyzed by BMDP statistical software. Nonparametric Friedman tests and the Mann-Whitney-"U" test were applied, and a p value below 0.05 was considered significant. Data were presented as mean ± one standard deviation.

Results

Serum levels of G-CSF, GM-CSF and IL-3 as well as absolute neutrophil counts (ANC) are shown in Table I.

Table I: G-CSF, GM-CSF, IL-3 Levels and Absolute Neutrophil Counts (ANC)

n = 25	1st Day	2nd Day	5th Day
G-CSF (pg/ml)*	191 ± 90	128 ± 20	112 ± 17
GM-CSF (pg/ml)**	9.5 ± 1.9	10 ± 2.2	8.8 ± 1.8
IL-3 (pg-ml)**	110 ± 35.5	138 ± 58	138 ± 74
ANC (10^9/L)	14 ± 3.9	7.5 ± 2.2	4.5 ± 1.6

* Adult levels were reported to be less than 30 pg/ml by the manufacturer.
** Adult levels were reported to be nondetectable by the manufacturer.

Mean serum concentrations of G-CSF were high on the first day of life and gradually decreased on the second and fifth days (Table I). A statistically significant correlation ($p < 0.05$) was found between G-CSF levels and ANC, but no correlation between any other parameters could be found. Furthermore, the percentage of the decrease between the first and second, second and fifth, and first and fifth days of life in G-CSF levels correlated significantly with the percentage of decrease in ANC (Fig. 1). Friedmann tests were used for analysis; the p value was 0.0027 and the Kendall coefficient of concordance was 0.3600 for ANC and G-CSF values on the first versus second days. Similar calculations for ANC and G-CSF (first versus fifth days) showed $p = 0.0001$, and the Kendall coefficient of concordance was 0.5776. Calculations for the second and fifth days showed $p = 0.0027$ and the Kendall coefficient of concordance value to be 0.3600.

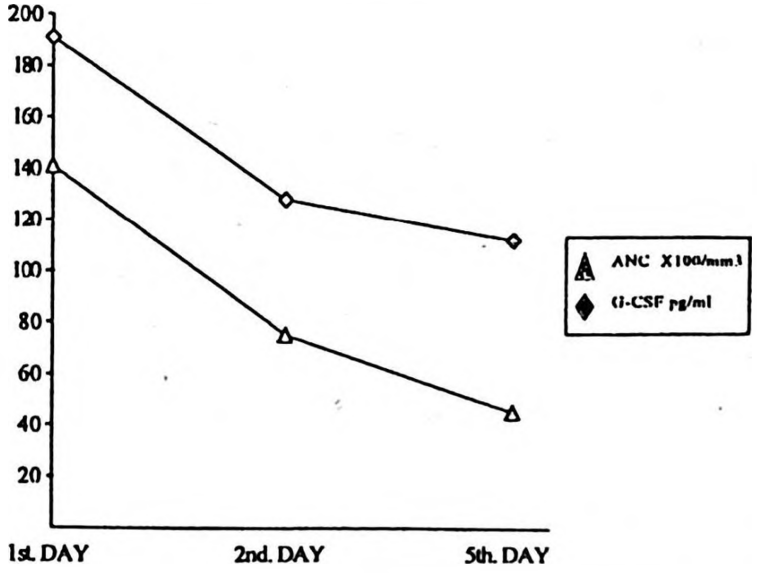

Fig. 1: G-CSF and absolute neutrophil values.

GM-CSF levels were also high, though not as striking as the others, on the first day of life, stayed at high levels on the second day, and decreased slightly on the fifth day. IL-3 levels were high on the first day of life and remained at high levels on the second and fifth days (Table I).

The mean values of the G-CSF receptor-positive neutrophil percentage was found to be 70 ± 4 percent (range 64-78%, n = 16) in cord blood, and it was not different from that in the peripheral blood of adult controls (77 ± 5%, range 69-83%, n = 10 $p > 0.05$). Mean channel numbers also did not differ significant (cord blood = 25.3 ± 1.2, adults = 23.6 ± 0.9, $p > 0.05$).

The GM-CSF receptor-positive neutrophil percentage (89 ± 4%, range: 82-95%, n = 16) in cord blood was not different from that in adult controls (86 ± 4%, range: 76-90%, n = 10, $p > 0.005$). The mean channel values were also similar in cord blood (25 ± 1.5) and adult controls (mean = 24.5 ± 1.0 $p > 0.05$).

Discussion

We observed a peak in the absolute neutrophil counts on the first day and a rapid decrease thereafter, as previously reported[3-5]. This decrease significantly correlated with the decrease in G-CSF levels (Table I). Our study shows that neonates have high levels of G-CSF at birth. Although a rapid decrease was observed on the second and fifth days, it was still higher than the adult levels (Table I). G-CSF levels correlated significantly with absolute neutrophil counts; the percentage of decrease in G-CSF correlated significantly with the percentage of decrease in ANC (Fig. 1). Our findings suggest that significant changes in the levels of growth factors may be the cause of this phenomenon. The lack of correlation between the ANC and the GM-CSF or IL-3 levels could be explained by their relatively wider spectrum of activity on the progenitor cells.

Russel et al.[11] also reported high G-CSF levels on the first day of life and a rapid decline by days 4-9. However, they found no correlation between the G-CSF levels and neutrophil counts. Yurdakök et al.[12] reported elevated G-CSF and GM-CSF levels in preterm neonates within 12 hours after birth. Laver et al.[13] and Ishii et al.[14] also reported significantly high G-CSF levels as compared to adults. Gessler et al.[15] found elevated G-CSF levels which peaked at seven hours after birth and declined on the fourth to seventh days. Our results corroborate these previous studies, but the correlation between the G-CSF levels and absolute neutrophil counts have not been reported before.

In contrast, Schibler et al.[16] and Cairo et al.[17] showed that the G-CSF production and gene expression of monocytes after stimulation was low in preterm and term newborns as compared to adults. Schibler et al.[16] found increased G-CSF serum levels in non-neutropenic newborns (values were very similar to ours).

Interestingly they showed that G-CSF serum concentrations were not elevated in seven neutropenic neonates, a finding which is in contradistinction to Russel et al.'s.[18] findings of significantly elevated pretreatment G-CSF levels in 12 neutropenic preterm neonates.

Medlock et al.[19] reported that maternally administered G-CSF crosses the placenta (although levels were 1000-fold lower than in the mother), and stimulates fetal rat granulopoiesis. (The G-CSF levels returned to baseline levels in 12 hours). It is possible that the increased levels of G-CSF on the first day, observed in our study and in previous studies, might have a maternal or a placental origin, as suggested by Ishii et al.[14], but its persistence on the second and fifth days is in favor of endogenous production. [The half life of serum G-CSF was reported to be 4.4 hours[20]]. Further studies are needed in order to clarify the production site and rate of G-CSF during increased demand in the early days of life.

There is only one study on IL-3 levels in the neonatal period, conducted by Meister et al.[21]. They found elevated levels of IL-3, which persisted for a month, in both premature and mature newborns. Our results confirm this study. Cairo et al.[17] reported decreased IL-3 production of stimulated mononuclear cells in newborn infants.

We observed high GM-CSF levels in neonates, which did not change during the observation period. High GM-CSF levels have been reported previously[12, 13], although Cairo et al.[22] found nondetectable levels of GM-CSF in the sera of cord and adult peripheral blood, and decreased stimulated GM-CSF production of mononuclear cells and GM-CSF gene expression in term newborns. English et al.[23] also reported diminished GM-CSF production of mononuclear cells in the neonatal period. Using an ELISA kit, Meister et al.[21] found low concentrations of GM-CSF in 38 percent of premature and 53 percent of mature neonates. These discrepancies may be explained by the maternal or placental origin of these colony-stimulating factors. It is also possible that the mononuclear cells may not be the main producers of GM-CSF in the neonatal period. Since our knowledge of the physiologic levels of these colony-stimulating factors and their biologic relevance is very limited, the high serum levels of these factors, observed in our study and reported previously, may still be insufficent for the neonatal period.

Laver et al.[13] and Christensen et al.[1, 24] showed that cord blood had increased numbers of circulating hematopoietic progenitors, but the data obtained in rats indicated that neonates might have a low committed progenitor pool per gram of body weight. The observations of high GM-CSF, G-CSF and IL-3 levels and increased proliferative rates of hematopoietic progenitors indicate that the neutrophil production rate is high even in a steady state condition in neonates. This might be a physiological adaptation mechanism to extrauterine life. As Laver et al.[13] suggested, limited numbers of progenitors proliferating at maximal capacity might be unable to respond to the increased demand, and this progenitor pool could become exhausted even in the presence of high levels of growth factors[1, 24, 25].

In our study, GM-CSF and G-CSF receptor-positive neutrophil percentages were found not to be different in cord blood when compared with those in the peripheral blood of adult controls. The receptor densities, assessed by mean fluorescence channel number, were also similar in newborn and adult neutrophils. Neonatal GM-CSF receptor number and affinity were found to be normal by Cairo et al.[22]. Browmeyer et al.[26] also showed that adult and term cord blood myeloid progenitors respond similarly to GM-CSF stimulation, which indirectly shows that neonatal neutrophils have normal receptors and respond to GM-CSF. Our study confirms these reports and shows that G-CSF receptor levels in newborns are also not different from adults. Cairo et al.[17], using a different method, also showed that G-CSF receptors were equal both in number and affinity in term newborns as compared to adult mature effector neutrophils. Our and previous data on these receptors were obtained from uninfected neonates. It has been shown that G-CSF, GM-CSF, IL-3, TNF and bacterial lipopolysaccharide down-modulate the G-CSF receptor expression[8], so further studies are needed on the expression of these receptors in newborns with sepsis.

TNF-alpha and gamma-interferon increase during infections, and they have suppressive effects on myelopoiesis[27, 28]. Their possible effects on neutropenia in septic newborns have not yet been fully investigated. Further studies on the effects of these growth factors, their metabolism and relations with other cytokines during severe infections, are needed.

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