Granulocyte colony-stimulating factor receptor expression on neutrophils of term and preterm neonates with and without signs of infection

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Abstract Neutrophils are an essential component of the human host defence system against infection. Recombinant human granulocyte colony-stimulating factor induces neutrophilia and enhances effector functions of mature neutrophils. Since the biological effects of granulocyte colony-stimulating factor (G-CSF) are mediated by its receptor, we investigated the expression of G-CSF receptor on the surface of neutrophils of term and preterm neonates (n = 22) with and without signs of infection and of healthy adults (n = 13) by flow cytometry. In healthy adults, the percentage of neutrophils expressing G-CSF receptor was higher compared to cord blood of term and preterm neonates (87% vs 53%, P < 0.05). Between 2 and 32 h of life, neonates with signs of infection showed lower values of G-CSF receptor expression compared to neonates without signs of infection (32% vs 54%, P < 0.05). No correlation was detectable between expression of G-CSF receptor and gestational age.

Conclusion Expression of granulocyte colony-stimulating factor receptor on neutrophils is lower than in adults. This may adversely affect granulopoiesis and neutrophil function during the neonatal period. Moreover, granulocyte colony-stimulating factor receptor expression seems to be down-regulated during neonatal infection.

Key words Granulocyte colony-stimulating factor receptor · Flow cytometry · Neonates

Introduction The human neonate is uniquely susceptible to severe and overwhelming bacterial infections [25]. One of the most important deficits in the neonatal host defence system seems to be a quantitative and qualitative deficiency of the myeloid and the phagocytic system [6, 8, 12, 26]. Granulocyte-colony stimulating factor (G-CSF) regulates the survival, proliferation, and differentiation of haematopoietic progenitor cells committed to the neutrophilic granulocyte lineage and the function of mature neutrophils [1, 29]. Recently, serum concentrations of G-CSF have been evaluated in term neonates and children [7, 10, 13, 14]. During the 1st day of life, high levels of G-
CSF have been reported in term and preterm neonates and even higher levels in neonates with signs of infection. Since the biological activities of G-CSF are mediated by specific receptors on the surface of responsive cells [3], the expression of G-CSF receptor on neonatal neutrophils seems crucial for the optimal G-CSF response. In the present study we focussed on the analysis of G-CSF receptor expression in term and preterm neonates with and without signs of infection.

Methods

Subjects and blood samples

The study population consisted of 22 term and preterm neonates and 13 healthy adults. Umbilical cord blood (0.5 ml) was collected immediately after delivery and two peripheral blood samples for flow cytometric analysis (0.5 ml) were taken in conjunction with routine blood analysis by venipuncture during the first 5 days of life. Blood was collected into K-EDTA coated tubes (Greiner, Nürtingen, Germany) and immediately processed.

Neonates were classified into two groups: Group 1, neonates with signs of infection comprised six neonates (gestational age 31 ± 6 weeks, range 26–40 weeks, four preterm, two term neonates) with signs of infection comprising six neonates (gestational age 31 ± 6 weeks, range 26–40 weeks, four preterm, two term neonates). Infection was presumed in a clinically symptomatic child in case of serum C-reactive protein levels >10 mg/l and immature-to-total neutrophil ratio (I/T) ≥0.2 or total neutrophil cell (TNC) counts <1000/µl during the first 48 h of life (corroborated at least once in every child) [17, 21]. TNC count was calculated for each blood cell count by multiplying the total white blood count (corrected by nuclear red cells) by the percentage of polymorphonuclear leucocytes and band forms. An I/T ratio was calculated for each TNC count as the ratio of immature neutrophils (band forms, metamyelocytes, and myelocytes) divided by TNC counts in the peripheral blood. A band was defined as a neutrophil in which the width of the isthmus was greater than 50% of the diameter of the widest nuclear lobe.

Group 2 comprises neonates without signs of infection, as outlined for group 1. This group consisted of 16 neonates (gestational age 33 ± 4 weeks, range 26–40 weeks, preterm n = 9, term neonates n = 7).

The study was approved by the medical ethics committee of the University of Tübingen and informed consent of the parents of each child was obtained.

Analysis of G-CSF receptor

Expression of G-CSF receptor on neutrophils was measured immediately after sample collection using a flow cytometric technique. Because levels of cytokine receptor expression are generally low, three-layer immunofluorescence was used to identify cells expressing G-CSF receptor [30]. Briefly, whole blood (0.05 ml) was mixed with recombinant human granulocyte colony-stimulating factor (rhG-CSF) (Neupogen, Amgen, Thousand Oaks, USA) to a final concentration of 10 pg/µl. After an incubation period of 15 min at 4°C and washing with PBS (CELL-Wash, Becton Dickinson, New Jersey, USA), anti-human granulocyte colony-stimulating factor antibody (anti-rhG-CSF, purified goat IgG, R&D Systems, Minneapolis, USA) was added. After incubation and washing, human immunoglobulin (Polyglobin N, Bayer, Leverkusen, Germany) was added and incubated for 15 min followed by the addition of fluorescein-conjugated Fc fragment specific affinity pure rabbit anti-goat IgG (Jackson Immuno Research, West Grove, USA). Thereafter, CD33 (Leu-M9 PE, Becton Dickinson) and CD16 (3G8 Tri-Color, MHC1606 Caltag Laboratories, San Francisco, USA) were added and incubated for 15 min at room temperature in the dark. As a negative control, all steps were performed in exactly the same way, however, without adding anti-rhG-CSF. Antibodies were used at saturating concentrations. Isotype control was performed with every blood sample (Simultest Control y1/1 IgG1 FITC, IgG1 PE, Becton Dickinson). After lysis of the erythrocytes using FACS-Lysing solution (Becton Dickinson), cells were analysed by flow cytometry.

Flow cytometry analysis

We used a standard flow cytometer (FACScan, Becton Dickinson, San Jose, Ca.). An amount of 15,000 cells was acquired. A two parameter light scatter dot plot was created and a software gate was set around the neutrophil population (Fig. 1A). The gated cells were analysed in respect of fluorescence intensity. A negative G-CSF receptor control was used to set a threshold (marker M1) allowing less than 5.0% of cells to remain positive (Fig. 1B). The percentage of cells in that positive region as well as their relative fluorescence intensity was analysed. The lysys II software (Becton Dickinson) was run on a HP9000 computer (Hewlett Packard). A daily control of fluorescence calibration was derived by analysing fluorescent latex beads (CaliBRITE Beads, Becton Dickinson).

Statistical analysis

Statistical analysis was performed using the Mann–Whitney test (for unpaired continuous variables, two-tailed P value). Non-