

Monocyte switch in neonates: high phagocytic capacity and low HLA-DR expression in VLBWI are inverted during gestational aging

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Pre-term neonates are at high risk to develop early-onset sepsis which possibly is caused by an immature immune system. Monocytes play a pivotal role as professional phagocytic and antigen-presenting cells in the innate immunity. In the present study, we investigated in monocytes from cord blood the expression of human leukocyte antigen (HLA)-DR as a marker for antigen-presenting capability, the expression of the high-affinity receptor for IgG (FcγRI/CD64), and the capacity to phagocytize non-opsonized *Escherichia coli*. We compared 70 infants in three groups according to their gestational age (group I: 20 very low birth weight infants (VLBWI), 24–31 weeks of gestation; group II: 25 pre-term infants, 32–36 weeks of gestation, and group III: 25 term neonates). The expression of CD64 as well as the phagocytic capacity of monocytes from cord blood were highest in VLBWI ($p < 0.05$ and $p < 0.01$, respectively). In contrast, HLA-DR expression was significantly ($p < 0.05$) diminished in VLBWI, which possibly leads to a reduced antigen-presenting capacity. We conclude that monocytes have different functional properties during gestational aging, which perhaps participate in the high incidence of infections in VLBWI.

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Premature newborns are particularly at a high risk of life-threatening sepsis possibly because of an immature immune system. The cellular and humoral functions play a decisive role for the immunological effectiveness during the first hours of life. It has been previously reported that the immune response of the pre-term neonates has not the same potency than term neonates (1, 2). Especially the first-line immune response in very low birthweight infants (VLBWI; birthweight below 1200 g) is thought to be reduced (3, 4).

Monocytes and macrophages belong to the first-line immune system. They are widely distributed in normal tissue, and take part in both, specific and non-specific immunity against bacterial, viral, and fungal infection. Monocytes express Fc receptors of three distinct classes of

FcγR on human leukocytes, termed FcγRI/CD64, FcγRII/CD32, and FcγRIII/CD16 (5). They are very effective in binding, internalizing, and destroying opsonized particles via these receptors. Monocytes also express high levels of major histocompatibility complex (MHC) class II molecules, which play a crucial role in antigen presentation to CD4⁺ T cells (6). In adult patients it has been demonstrated that reduced monocyte human leukocyte antigen (HLA)-DR expression during infection and sepsis is linked to recovery and mortality rates (7, 8). In VLBWI reduced levels of HLA-DR are predictive of early onset sepsis (9).

As only little is known about monocyte development dependent on gestational aging we investigated the expression of FcγRI, the phagocytic capacity as well as the expression of

HLA-DR as a marker for the capability to present bacterial antigen in cord blood monocytes from VLBWI to term neonates.

Material and methods

The study was performed at the Department of Pediatric Surgery, Division of Surgical Research Laboratories, and at the Department of Neonatology and Intensive Care, University of Vienna within a time period of 1.5 yr. This study was conducted under the auspices of the Ethical Committee of the University of Vienna, and informed consent was obtained from each patient before enter of the study.

The neonates enrolled into the study were classified into three groups. Group I: very low birth weight infants aged between the 24th and the 31st week of gestation with a birth weight under 1500 g (1138 ± 285 g). Group II: pre-term neonates aged between the 32nd and the 36th week of gestation with a birth weight from 1500 to 2800 g (2261 ± 449 g). Group III: term neonates aged between the 37th and the 42nd week of gestation, weighing between 2800 and 4300 g (3452 ± 412 g). There was a close correlation (correlation coefficient 0.935) between gestational age and birth weight (data not shown). Clinical data were recorded by a standard protocol including gestational age, birth-weight, chorioamnionitis, and early rupture of membranes.

Cord blood was collected < 15 min after birth. Samples were obtained aseptically by squeezing or puncturing of the cord. 100 μ L of whole blood was stained with anti-CD14 mAb (My4 Cy5; Coulter, Hialeah, FL, USA), anti-CD64 FITC (Medarex, West Lebanon, NH, USA) and anti-HLA-DR (PE; Becton Dickinson, San Jose, CA, USA). Another 100 μ L of whole blood was stained with isotype control mAbs (Immunotech, Marseille, France). After incubation for 30 min on ice red blood cells were lysed. Subsequently cells were washed three times in Hanks' balanced salt solution (HBSS), and the expression of HLA-DR and of CD64 on CD14⁺ cells was measured by flow cytometry (Coulter EPICS-XL; Coulter). At least 2.5×10^3 of CD14⁺ cells were counted. Results are given as mean channel fluorescence intensity (MCF).

Phagocytosis was determined by incubating 100 μ L of whole blood for 15 min at 37°C with non-opsonized, FITC-conjugated *E. coli* (Orpegen, Heidelberg, Germany). Subsequently cells were stained with anti-CD14 mAb (My4 Cy5), and red blood cells were lysed (Multi-Q-Prep; Coulter). Cells were washed three times in HBSS.

The MCF of 2.5×10^3 CD14⁺ phagocytic cells was determined by flow cytometry.

Data are presented as mean $\pm$ standard deviation. Statistical analysis was performed using the Student's *t*-test. For multiple comparisons, One-way analysis of variance (ANOVA) with Tukey-HSD *post hoc* test was applied. Regression analysis was performed using the SPSS software package (SPSS for Windows, Release 8.0.0; SPSS, Chicago, IL, USA). A probability of $p < 0.05$ was considered statistically significant.

Results

A total of 75 neonates were included in the study, 25 neonates per group. Five of the VLBWI (group I) developed early-onset sepsis and were therefore excluded from the study. The remaining 70 neonates showed no clinical signs of infections in the first weeks of life.

As can be seen in Fig. 1, CD64 expression (black bars, $p < 0.05$) as well as phagocytosis (line, $p < 0.01$) decreased significantly (one-way ANOVA). Tukey-HSD *post hoc* test analysis for CD64 expression showed significant differences between groups I and II ($p < 0.05$) as well as between groups I and III ($p < 0.01$). *Post hoc* test analysis for phagocytosis showed significant differences between groups I and II ($p < 0.01$) as

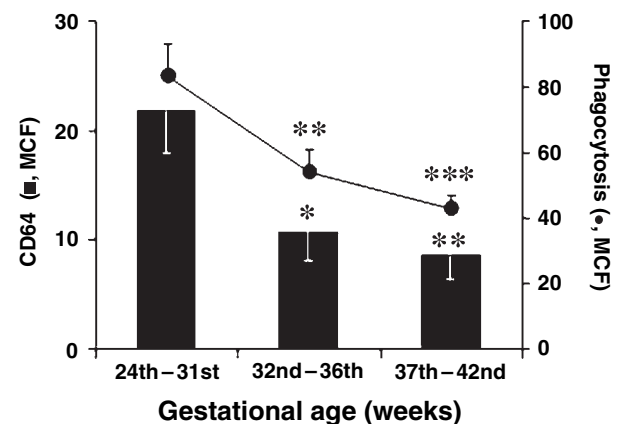


Fig. 1. CD64 expression (black bars) and *Escherichia coli* phagocytosis (line) of CD14⁺ monocytes from cord blood investigated by flow cytometry. CD64 expression and phagocytosis are given as mean channel fluorescence intensity (MCF). CD64 expression ($p < 0.05$) and phagocytosis ($p < 0.01$) were analyzed by ANOVA. Tukey-HSD *post hoc* test analysis for CD64 expression showed significant differences between groups I and II (* $p < 0.05$) as well as between groups I and III (** $p < 0.01$). *Post hoc* test analysis for phagocytosis showed significant differences between groups I and II (** $p < 0.01$) as well as between groups I and III (** $p < 0.001$). There were no significant differences between groups II and III.

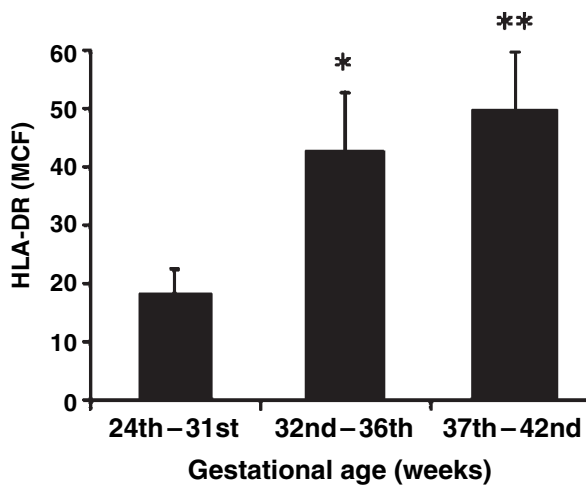


Fig. 2. HLA-DR expression of CD14s⁺ monocytes from cord blood investigated by flow cytometry and given as mean channel fluorescence intensity (MCF). HLA-DR expression ($p < 0.05$) was analyzed by ANOVA. Tukey-HSD *post hoc* test analysis showed significant differences between groups I and II (* $p < 0.05$) as well as between groups I and III (** $p < 0.01$). There were no significant differences between groups II and III.

well as between groups I and III ($p < 0.001$). There were no significant differences between groups II and III. In contrast monocyte HLA-DR expression increased significantly ($p < 0.05$, one-way ANOVA, Fig. 2). Tukey-HSD *post hoc* test analysis showed significant differences between groups I and II ($p < 0.05$) as well as between groups I and III ($p < 0.01$). There were no significant differences between groups II and III.

Discussion

In VLBWI the development of infectious complications is associated with a high morbidity and mortality. In the present study, we investigated the expression of HLA-DR as a marker of bacterial antigens, the expression of the high-affinity receptor for IgG/CD64 and the capacity to phagocytize non-opsonized *E. coli* dependent on gestational maturation. CD64 expression as well as phagocytosis were decreased whereas monocyte HLA-DR antigen increased from VLBWI to term neonates. These data indicate a transformation of the immature monocytic cell into a mature cell with highly specialized properties.

Phagocytic capacity in pre-term and term babies is widely discussed. However, there is currently no literature available describing monocyte phagocytic properties in dependency of gestational maturation. Phagocytosis is mediated by opsonins, especially by IgG. Previous

studies have shown that IgG transfer through the placental barrier starts predominantly up to the 32nd week of gestation (10). Therefore we divided the pre-term neonates into two groups. Although in our test system non-opsonized *E. coli* were used and the amount of free IgG determined from cord blood was reduced in the group of VLBWI (data not shown), we detected an increased phagocytic capacity. Possibly the elevated expression of CD64 leads to an improved recognition of opsonized *E. coli* independent of the amount of IgG bound on the particle. Interestingly it has been shown that an enhanced expression of CD64 on granulocytes is a highly specific indicator of neonatal sepsis (11).

In contrast in monocytes from cord blood from VLBWI we found a decreased HLA-DR expression when compared with term neonates. A reduction of monocyte HLA-DR cell surface antigen has been previously correlated with the appearance of infectious complications (8). Recently we demonstrated that VLBWI developing early onset sepsis have a massively impaired phagocytic capacity and a diminished HLA-DR expression (9). However, taking into account that the HLA-DR antigen is indispensable to present bacterial antigen to CD4⁺ lymphocytes, a reduction in HLA-DR possibly causes an insufficient T-lymphocyte response. Therefore, even a high phagocytic capacity is not necessarily combined with a sufficient first-line immune response. However, a higher expression of HLA-DR in the later gestational age does not necessarily imply a specific higher capacity to present bacterial antigens.

To date, monocyte development dependent on gestational age is not yet clear. For hematopoietic progenitor cells the variability of cell surface antigen expression is dependent on gestational aging (12). The percentage as well as the absolute number of CD34⁺ cells detectable in human cord blood were significantly higher in very pre-term fetuses compared with pre-term and term newborns. The highest proliferative activity was found at the lowest gestational age, and progressively decreased with advanced gestational aging. These data support our findings as a part of the hematological system matured from non-specific properties to specialized functions.

In conclusion, monocytes have varying physiological functions during gestational aging. Although a high phagocytic capacity exists in VLBWI, expression of HLA-DR antigen is low, which possibly bears a role in the high incidence of infectious complications in VLBWI. However, in mature newborns functional properties are inverted.

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