

Gestational Age Related Maternal-Fetal-Neonatal Humoral Immunity

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ABSTRACT

We studied the total complement activity (CH50), the hemolytically effective molecules of complement components (C1q, C1, C2, C3 and C5), immunoglobulin (Ig) A, M, G, and E, and circulating immune complexes (CIC) in 44 mothers (venous blood) and their prematurely born newborn infants (cord blood obtained shortly after birth, venous blood on day 3 to 4 of life). All the study infants were singleton births, delivered vaginally and there was no evidence of maternal infection or asphyxia. Gestational age ranged from 28 to 32 weeks (Group 1) and 33 to 36 weeks (Group 2).

Mothers in Group 1 had lower IgM levels (0.86 ± 0.30 g/L, $P < 0.037$) as compared to Group 2. Lower gestational age was associated with decreased cord blood IgG (5.7 ± 0.60 g/L, $P < 0.006$), IgA (0.26 ± 0.17 g/L, $P < 0.01$) and CH50 (43.5 ± 33.2 U/mL, $P < 0.018$). In preterm neonates with a lower gestational age (Group 1) the IgA, IgM, CIC and CH50 increased on day 3 to 4 of life as compared to cord blood, whereas the IgG remained low. Additionally, the IgA ($P < 0.011$), IgM ($P < 0.013$), and CIC ($P < 0.044$) levels as well as the number

of effective molecule of C5 ($P < 0.04$) exceeded those of Group 2.

In prematurely born infants of ≤ 32 weeks gestation, the decreased total complement activity and the major immunoglobulin class levels seen at birth (cord blood) were followed by complement activation, increased circulating antibody (except IgG) and immune complexes by day 3 to 4 of life. This may characterize activation of the complement system, which in the presence of low circulating IgG, in neonates with a lower gestational age, makes them vulnerable to complement-mediated organ damage. However, a postnatal activation of the cascade or a stimulation of synthesis of other factors causing an increase of the humoral factors cannot be excluded.

INTRODUCTION

Deficiency of humoral immunity exerts considerable influence on the prematurely born neonate's susceptibility to infection,^{1,2} and other morbidity and mortality related to prematurity.³ The essential components of humoral immunity are complement and circulating immunoglobulin, and with the exception of immunoglobulin G do not cross the placenta and are produced by the fetus in the early stages of gestation.^{4,6}

Complement and immunoglobulin have been studied extensively with

Table 1. Comparison of Maternal-Fetal-Neonatal Humoral Immunity in Group 1 (n=14).

	Maternal	Fetal	Neonatal	P value*	P value†	P value‡
C1q (x10 ¹²)	133.8±65.0	86.8±55.0	85.6±35.7	<0.01	<0.01	NS
C2 (x10 ¹²)	88.2±34.5	33.5±34.0	54.0±31.5	<0.01	<0.01	NS
C3 (x10 ¹²)	135.4±51.7	85.1±54.2	96.9±45.1	<0.01	<0.01	NS
C4(x10 ¹²)	122.8±53.1	66.4±60.5	79.8±48.5	<0.01	<0.01	NS
C5 (x10 ¹²)	105.2±28.9	77.8±21.2	88.5±29.3	<0.01	<0.01	NS
C50 (U/mL)	126.4±42.9	43.5±33.2	69.0±31.2	<0.01	<0.01	<0.01
IgA (g/L)	1.04±0.64	0.26±0.17	0.68±0.38	<0.001	<0.01	<0.01
IgM (g/L)	0.86±0.30	0.17±0.16	0.58±0.31	<0.001	<0.01	<0.01
IgG (g/L)	7.27±1.54	5.70±0.60	5.89±1.41	<0.01	<0.01	NS
IgE (IU/L)	29.0±21.8	1.65±1.16	2.62±1.41	<0.01	<0.01	NS
C1C (g/L)	0.14±0.04	0.03±0.03	0.05±0.02	<0.001	<0.001	0.035

*Comparison between maternal and fetal levels; †comparison between maternal and neonatal levels; ‡comparison between fetal and neonatal levels

respect to gestational age, infection, and other medical conditions.⁷⁻¹¹ Gestational age dependent reduction of complement activity and its regulation capacity has been previously reported in prematurely born neonates.¹²⁻¹⁶ However, none of the previous studies investigated the influence of gestational age on the functional activity of complement components, circulating immunoglobulin, and immune complex levels in the mothers and their prematurely born neonates.

The objective of this study was to investigate the humoral immune status of the mothers and their prematurely born neonates during the early days of life.

MATERIALS AND METHODS

This study was performed at the Belarusian Mother and Child Care Research Institute, Hospital #7, Minsk, Belarus with permission from the institutional scientific committee and all their requirements were complied with. Secondary data analysis was approved by the University of Medicine and Dentistry of New Jersey-Robert Wood Johnson Medical School Institutional Review Board.

A total number of 44 mothers and their prematurely born infants were studied. The inclusion criteria were gestational age between 28 to 36 weeks (assigned using maternal menstrual dates and confirmed by neonatal clinical assessment), no evidence of maternal infection during pregnancy and delivery, singleton birth, vaginal delivery, rupture of membranes for less than 18 hours, no laboratory evidence of infection, and Apgar score greater than 3 at 1 minute.

Approximately 1.5 milliliters each of maternal, cord, and neonatal blood (day 3 to 4 of life) was collected, centrifuged, and stored at -80° C until analysis. The classical pathway (CH50) and complement components C1q-C5 were measured using specific antibody/hemolysin coated sheep erythrocytes.¹⁷⁻¹⁹ The CH50 was expressed as the titer of CH50 units per mL that were a reciprocal of the dilution of complement, which lysed 50% of the sheep erythrocytes (U/mL). Activity of C1q-C5 was expressed by the quantitative determination of the number of effective molecules/mL (x10¹²) of complement components in the human serum. This was estimated by multiplication of the

Table 2. Comparison of Maternal-Fetal-Neonatal Humoral Immunity in Group 2 (n=30).

	Maternal	Fetal	Neonatal	<i>P</i> value*	<i>P</i> value†	<i>P</i> value‡
C1q (x10 ¹²)	123.8 ± 56.3	80.7± 49.9	80.9± 43.4	<0.01	<0.01	NS
C2 (x10 ¹²)	115.3 ± 45.9	51.8± 30.0	62.9± 21.1	<0.01	<0.01	NS
C3 (x10 ¹²)	153.9 ± 55.2	86.8± 47.2	89.1± 36.9	<0.01	<0.01	NS
C4 (x10 ¹²)	122.8 ± 65.6	84.8± 57.8	80.7± 47.1	<0.01	<0.01	NS
C5 (x10 ¹²)	100.5 ± 29.3	65.5± 26.1	70.6± 24.3	<0.01	<0.01	NS
CH50 (U/mL)	130.6 ± 45.5	82.2± 43.8	73.7± 24.5	<0.01	<0.01	NS
IgA (g/L)	1.26 ± 0.81	0.43± 0.23	0.40± 0.31	<0.01	<0.01	NS
IgM (g/L)	1.41 ± 0.87	0.25± 0.15	0.30± 0.24	<0.01	<0.01	NS
IgG (g/L)	9.46 ± 3.54	8.34± 3.14	7.94± 1.78	NS	NS	NS
IgE (IU/L)	27.1 ± 19.5	1.92± 1.57	2.83± 2.01	<0.01	<0.01	NS
CIC (g/L)	0.12 ± 0.08	0.03± 0.02	0.02±0.02	<0.01	<0.01	NS

*Comparison between maternal and fetal levels; †comparison between maternal and neonatal levels; ‡comparison between fetal and neonatal levels.

total number of erythrocytes and number of lysed erythrocytes using the equation $z = -\ln(1-y)$, where y is number of lysed erythrocytes.

The concentration of the 3 major classes of immunoglobulin (Ig) A, M, and G was determined using commercially available mono-specific antiserum by the single radial immunoassay method.²⁰ The ABBOTT solid-phase enzyme immunoassay was used to measure total serum immunoglobulin E (IgE) in human serum and plasma (IU/mL) in the dynamic range of 0.5 to 200 IU/mL. Circulating Immune Complex (CIC) was measured by the polyethylene glycol precipitation test.²¹

To investigate the influence of gestational age on maternal-fetal and neonatal complement activity, circulating immunoglobulin and immune complex levels, the data was stratified into 2 gestational age categories, 28 to 32 weeks (Group 1, n=14), and 33 to 36 weeks (Group 2, n=30).

Statistical analysis was performed using STATISTICA software (Statistica for Windows, 1984-1994, StatSoft, Inc.). Continuous data is presented as Mean (S) ± Standard Deviation (SD). The

Mann-Whitney U and the t test were used to analyze the data. All tests were two-sided.

RESULTS

In both groups of newborn infants, the total hemolytic activity (CH50), number of effective molecule of classical pathway (C1q-C5), immunoglobulin (IgM, IgA, and IgE), and circulating immune complex (CIC) levels were significantly lower as compared to their mothers, whereas the IgG was lower only in the neonates of Group 1 (Table 1 and 2). The preterm neonates of Group 1 had increased IgA, IgM, CIC, and CH50 during the first 3 to 4 days of life (Table 1). The preterm neonates of Group 2 showed no change in the studied immunological parameters (Table 2).

Gestational age stratification of the maternal-fetal-neonatal immunological parameters showed that Group 1 mothers had lower IgM levels than Group 2 (0.86±0.30 g/L, $P<0.04$). Figure 1 presents a comparison of the CH50, IgG, IgM, and IgA levels in the cord blood and on day 3 to 4 of life. In the cord blood, decreased IgG ($P<0.006$), and IgA ($P<0.01$) levels, and reduced CH50

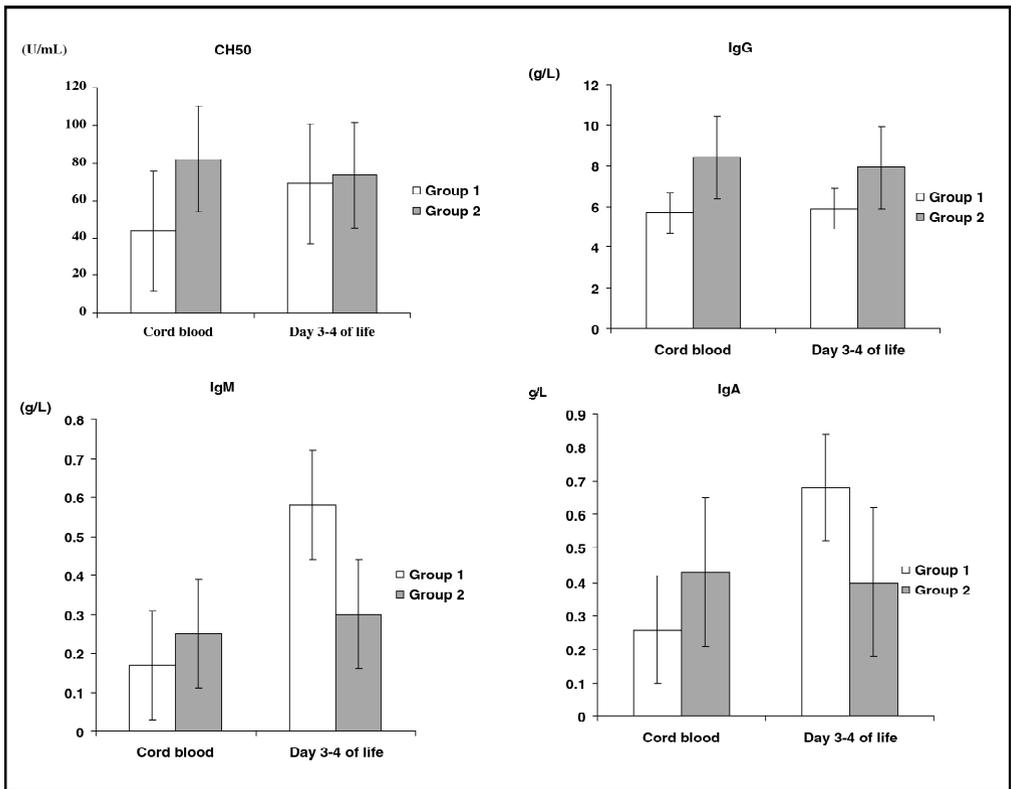


Figure 1. Comparison of CH50, IgG, IgM, and IgA in the cord blood and on day 3 to 4 of life based on gestational age groups

($P < 0.018$) functional activity was found in Group 1 as compared to Group 2 neonates. There were no significant differences in cord blood C1q-C5, IgM, IgE, and CIC levels between these 2 groups. On day 3 to 4 of life, IgA ($P < 0.011$), IgM ($P < 0.013$), and CIC ($P < 0.044$) levels as well as the number of effective molecule of C5 ($P < 0.04$) were higher in Group 1 as compared to Group 2 neonates. The quantity of hemolytically effective molecules C1q, C2, C3, and C4, CH50 and IgE levels were similar in the 2 groups.

There is correlation between gestational age and cord blood CH50 ($r = 0.32$, $P < 0.036$) and IgG ($r = 0.26$, $P < 0.05$) levels. On day 3 to 4 day of life, gestational age correlated with IgA ($r = -0.41$, $P < 0.005$), IgM ($r = -0.28$, $P < 0.01$), IgG ($r = 0.28$, $P < 0.044$) and CIC ($r = 0.35$,

$P < 0.032$) levels.

DISCUSSION

This study explores the association between gestational age, total complement hemolytic activity (CH50), quantity of hemolytically effective molecules of complement components (C1q-C5), immunoglobulin A, M, G and E, and circulating immune complexes (CIC) levels in maternal, cord, and neonatal serum. In accord with other publications,^{8,13} we found that with the exception of IgG in Group 2 neonates (GA > 32 weeks), the tested immunological parameters in both groups of prematurely born neonates were significantly lower than their mothers. Our findings are in concurrence with other studies^{12,13} that showed a direct association between

gestational age and CH50 in the cord blood of prematurely born neonates. Some studies have shown a relationship between some of the complement components and gestational age.^{22,23} However, in our study the gestational age related decrease in cord blood CH50 was not associated with a lowering of the quantity of hemolytically effective molecules of C1q-C5 complement components. The methodology we used measured the circulating functional integrity of the complement system, whereas these other studies^{22,23} analyzed complement protein levels. An experimental study²⁴ revealed the possibility of an independency between functional activity and complement component protein levels. Our findings may characterize in utero activation of the complement system (by complement consumption) in newborn infants with gestational age equal to or less than 32 weeks.^{8,25} However, a postnatal activation of the cascade or a stimulation of synthesis of other factors causing an increase of the humoral factors cannot be excluded. The increase of the humoral immune defence factors could also represent a compensatory response to the increased susceptibility of very preterm infants to infection.

Much of the literature implies the action of antibody and antigen-antibody complexes as well as aggregated immunoglobulin initiated complement activation via the classical complement pathway.¹⁴ Increased CIC and IgM levels during the first days of life confirm the enrolment of the antigen-antibody complexes in the mechanism of complement activation in the lower gestational age neonates. We found that in utero complement activation (cord blood) in preterm neonates of Group 1 results in an increased CH50 and C5 activity by day 3 to 4 of life. Despite the limited synthesis of IgA in newborn infants,¹⁴ we observed somewhat increased IgA levels

in neonates with a lower gestational age, which may reflect a compensatory mechanism to down-regulate complement activation.^{26,27}

The activation of complement component C5 that we found in the preterm neonates with a lower gestational age has also been implicated in the pathogenesis of adult respiratory distress syndrome.^{28,29} A poor capacity to control spontaneous complement activation because of the lower levels of circulating IgG,^{26,30} makes prematurely born neonates extremely vulnerable to complement-mediated damage of the lung and brain.¹⁴⁻¹⁶

With increasing survival of high-risk neonates, the involvement of the complement system and circulating immunoglobulin in the pathogenesis of lung and brain injury related to prematurity and the higher susceptibility to infection, has emerged as an area of priority in neonatal research. An improved understanding of the humoral immunity in preterm deliveries may offer future solutions to benefit neonatal outcome.

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