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Fetal B Lymphocyte Subpopulations in Normal Pregnancies

Key Words
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Abstract
In 190 pregnancies undergoing cordocentesis for prenatal diagnosis (n = 174) or elective caesarean section (n = 16), fetal peripheral blood B lymphocyte subpopulations were measured using a fluorescence-activated cell sorter (FACScan). The total number of B lymphocytes and polyreactive CD5+ B cells increased exponentially with gestation from respective means of 0.33×10^9/l and 0.25×10^9/l at 17 weeks to a plateau of 0.66×10^9/l and 0.54×10^9 at 36 weeks, remaining at that level thereafter. The number of mature CD10– and active CD23+ B lymphocytes increased linearly from a mean of 0.07×10^9/l and 0.11×10^9/l at 17 weeks to 0.24×10^9/l and 0.37×10^9/l, respectively, at 40 weeks. As expected, all B lymphocytes expressed the HLA-DR antigen from as early as 16 weeks gestation. These alterations in specific B lymphocyte subpopulations reflect the pattern of maturation and development of the fetal humoral immune system.

Introduction

During intra-uterine life, there is a linear increase in the total number of lymphocytes with gestation, and the values at term are similar to those in the adult [1]. However, neonates exhibit functional differences from adults in their humoral immune system, including limited antibody diversity and qualitatively reduced antibody responses [2–4]. Furthermore, the production of IgG and IgA in vitro is reduced even when neonatal B lymphocytes are stimulated by either polyclonal activators or adult T lymphocytes [5, 6]. These observations suggest that immaturity of fetal B lymphocytes as well as poor T lym-

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Table 1. List of the monoclonal antibody panel used to enumerate fetal B lymphocyte subpopulations, showing cluster designations or name (CD No./name), alternative nomenclature and reactivity/specificity

CD No./ name	Alternative nomenclature	Reactivity/specificity
CD19	Leu 12	pan-B lymphocyte marker
CD5	Leu 1	T and polyreactive B lymphocytes
CD10	CALLA	pre-B lymphocytes
CD23	Leu 20	activated B and T lymphocytes
HLA-DR		monocytes, B and activated T lymphocytes

phocyte cooperation might be the cause of the reduced perinatal response [7, 8]. The aim of the present study was to characterize the pattern of fetal B lymphocyte maturation with gestation using flow cytometry. The cell surface antigens examined were CD5, CD10, CD19, CD23 and HLA-DR, which characterize specific stages of B-lymphocyte maturation and aspects of B-cell function (table 1).

Patients and Methods

Fetal blood samples were obtained by cordocentesis from 174 pregnancies undergoing prenatal diagnosis at 17–40 weeks gestation. The indications for fetal blood sampling included: (i) fetal karyotyping for women of advanced age that booked late or for low maternal serum alpha fetoprotein (n = 29); (ii) fetal blood grouping in red blood cell isoimmunized pregnancies and where the fetus was subsequently found to be Coombs-negative (n = 5), and (iii) karytoyping for minor fetal malformations, such as choroid plexus cysts or hydronephrosis (n = 140). In all cases, the fetal abdominal circumference, blood gas values and haemoglobin concentration were within the appropriate reference range for gestation and the fetal karyotype was normal.

Additionally, cord blood samples were collected from normal pregnancies undergoing elective caesarean section at 38–40 weeks gestation (n = 16), which was performed either because of previous caesarean section and suspected cephalopelvic disproportion or for breech presentation. In all cases the infants were normal an their birth weight was above the 5th centile for gestational age.

The study was cross-sectional and in each case gestation was determined from the maternal menstrual history and confirmed by an ultrasound scan in early pregnancy. Cordocentesis was performed without maternal sedation or fetal paralysis and in all cases umbilical venous blood was obtained. Kleihaur-Betke testing confirmed that all blood samples contained only fetal blood.

Fetal blood samples (180 µl) were collected into 20 µl of isotonic edetic acid solution (0.5 mmol/l in 0.15 mmol/l sodium chloride) and the full blood count was determined using a Coulter S-Plus counter (Coulter Electronics, Luton, England). Blood films were stained by the May-Grünwald-Giemsa method for the differential cell count. Blood samples (250 µl) were collected into heparinized syringes for measurement of oxygen tension and pH (Radiometer ABL 330, Copenhagen, Denmark). Blood samples (0.5 ml) were also collected into heparinized syringes for enumeration of fetal lymphocyte subsets, which was performed on the day of sampling.

Flow Cytometry

Fluorescein-isothiocyanate (FITC) or phycoerythrin (PE) conjugated monoclonal anti-human antibodies (Becton Dickinson UK Ltd., Abingdon, England) were used for simultaneous two-colour determination of CD19+ with CD5+, CD10+, CD23+ and HLA-DR+ subpopulation (table 1).

The whole-blood method was used for staining of the cells with monoclonal antibody [9]. Fetal blood (50 µl) was incubated with 5 µl of the appropriate monoclonal antibody for 10 min. The blood was then incubated for a further 10 min in the dark, following

Table 2. Reference ranges for percentage and number of fetal B (CD19+) and CD19+CD5+ lymphocytes (mean and 95% confidence interval) with gestation (GA)

GA weeks	Total CD19+, %		Total CD19+, $\times 10^9$/l		CD19+CD5+, $\times 10^9$/l	
	mean	95% CI	mean	95% CI	mean	95% CI
16	11	0–23	0.28	0.03–0.61	0.23	0.00–0.51
18	13	0–26	0.36	0.08–0.71	0.28	0.01–0.61
20	14	1–27	0.42	0.13–0.78	0.34	0.06–0.68
22	16	3–29	0.47	0.18–0.85	0.40	0.11–0.74
24	17	4–30	0.52	0.21–0.91	0.44	0.15–0.79
26	17	4–30	0.56	0.25–0.96	0.48	0.18–0.84
28	18	5–31	0.60	0.27–1.01	0.51	0.20–0.88
30	18	5–31	0.63	0.30–1.04	0.53	0.22–0.90
32	17	4–30	0.65	0.31–1.07	0.55	0.23–0.92
34	17	4–30	0.66	0.32–1.08	0.55	0.23–0.92
36	16	3–29	0.66	0.32–1.09	0.54	0.23–0.91
38	14	2–27	0.66	0.32–1.08	0.53	0.21–0.90
40	13	1–26	0.65	0.30–1.08	0.50	0.19–0.88

addition of 2 ml of FACS lysing solution to lyse the red blood cells. After incubation, the cells were washed twice with phosphate-buffered saline containing 0.1% sodium azide.

Cytometric analysis was carried out using a fluorescence-activated cell sorter (FACScan) and Consort 32 software (Becton Dickinson). Samples were gated using forward angle and 90 light-scattering properties to exclude granulocytes, monocytes and platelets. Gated cells were analysed with CD14/CD45 (monocyte/lymphocyte marker) to ascertain that cells were lymphoid in origin. Control staining of fetal cells with anti-mouse monoclonal IgG_{2a}-PE/IgG_1-FITC was performed on each sample, and background readings of $< 1\%$ were obtained. A minimum of 5,000 cells were acquired in the lymphocyte fraction and analysed to calculate the percentages of each subpopulation. The absolute counts for each subset were calculated as products of the corrected lymphocyte count and the percentages of each lymphocyte subpopulation. The corrected lymphocyte count was derived from the white blood cell count measured by the Coulter counter and the lymphocyte differential count on the blood film.

Statistical Analysis

Regression analysis was used to determine the significance of any association between fetal lymphocyte subsets and gestational age. The residuals from linear regression were tested for normality. Abnormally distributed data was made Gaussian by logarithmic transformation. The regression data were used to calculate the reference range with gestation in the original units (mean and individual 95% confidence intervals.). To determine the reference ranges in the original units, the limits of the calculated reference range in logarithms were subjected to antilogarithmic transformation.

Results

With advancing gestation there was an exponential increase in the number of CD19+ and CD19+CD5+ cells (table 2, fig. 1). The number of CD19+CD5–, CD19+CD10– and CD19+CD23– lymphocytes did not change significantly with gestation (fig. 1–3). The number of CD19+CD10– and CD19+CD23+ cells increased linearly with gestation age (table 3, fig. 2, 3). All B lymphocytes expressed the HLA-DR antigen on their cell surface.

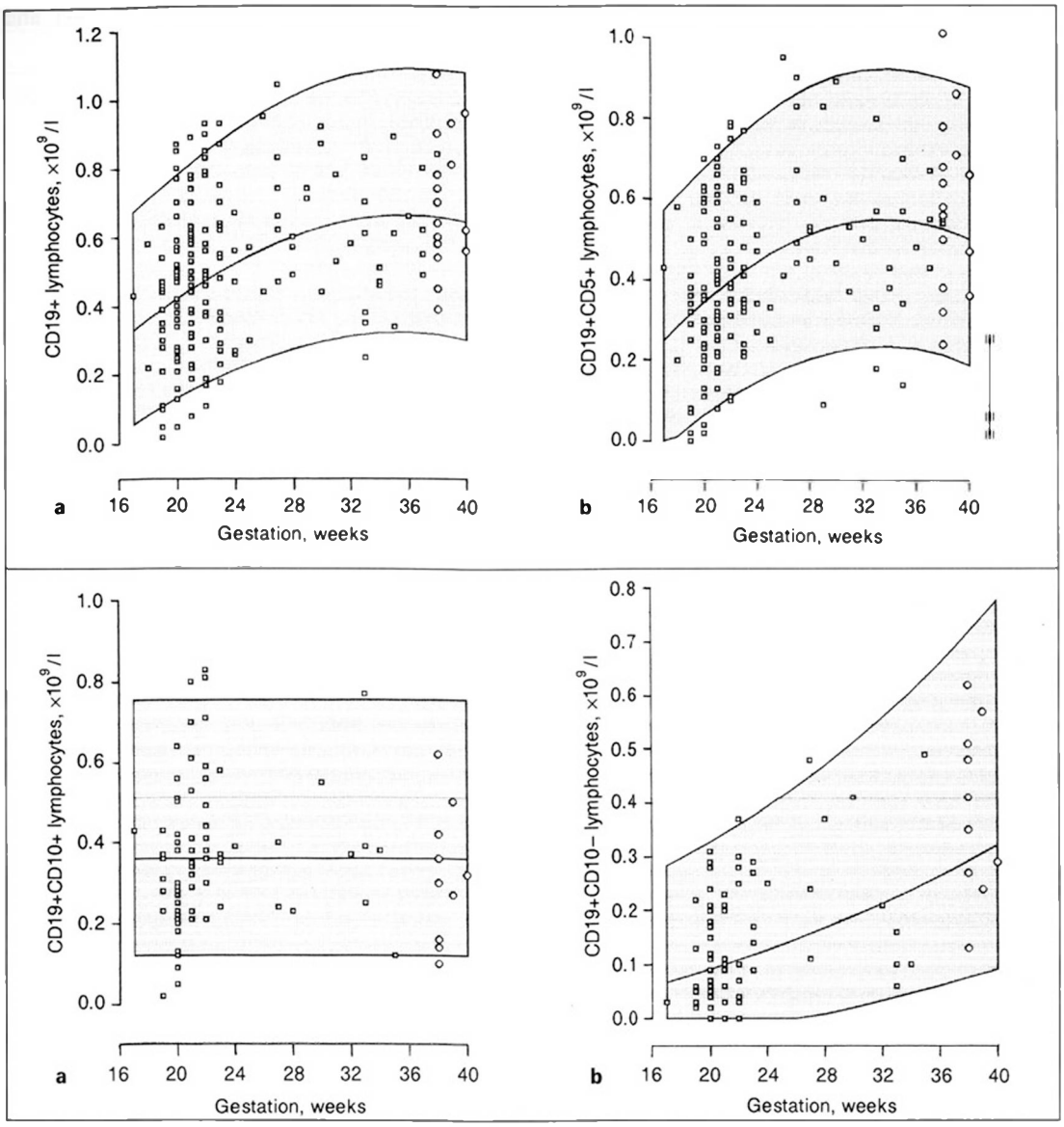

Fig. 1. Fetal CD19+ lymphocyte (**a**) and CD19+CD5+ lymphocyte (**b**) numbers plotted (cordocentesis □, caesarean section ○) as a function of length of gestation (CD19+ number: $p < 0.05$, $r = 0.414$; $n = 190$, CD19+CD5+ number: $p < 0.05$, $r = 0.384$; $n = 175$). The sloping lines are the mean, 2.5th and 97.5th percentile values. The vertical bar on the right represents the median and range for the CD19+CD5− cells (CD19+CD5− number: median = 0.06 × 10^9/l, 95% confidence interval = 0.02–0.25 × 10^9/l).

Fig. 2. Fetal CD19+CD10+ lymphocyte (**a**) and CD19+CD10− lymphocyte (**b**) numbers plotted (cordocentesis □, caesarean section ○) as a function of length of gestation (CD19+CD10+ number: median = 0.36 × 10^9/l. 95% confidence interval = 0.12–0.76 × 10^9/l, CD19+CD10− number: $p < 0.05$, $r = 0.229$; $n = 86$). The sloping lines are the mean, 2.5th and 97.5th percentile values.

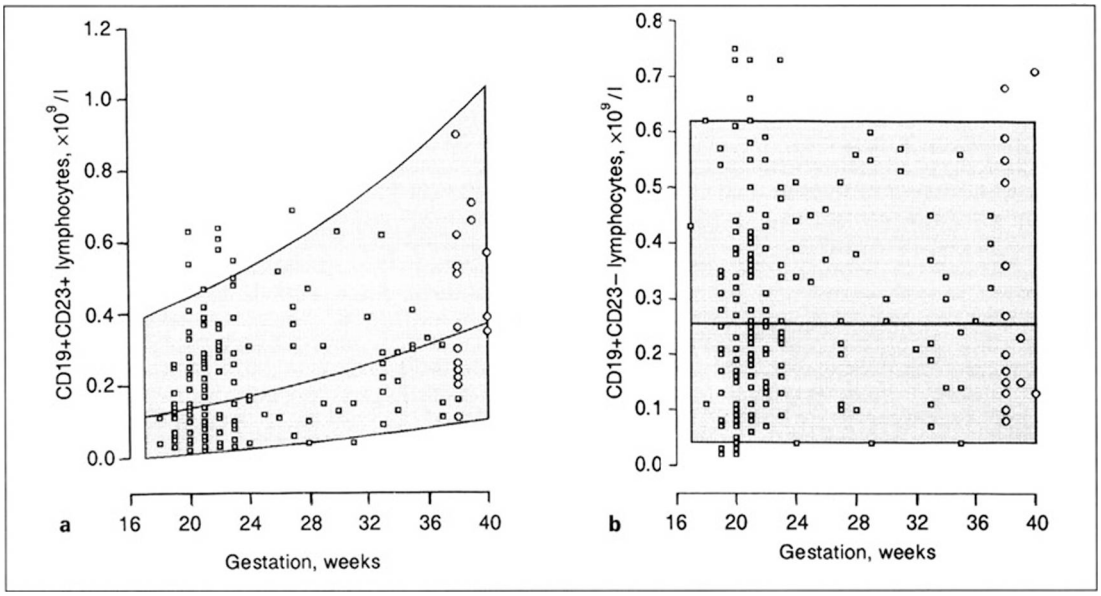

Fig. 3. Fetal CD19+CD23+ lymphocyte (**a**) and CD19+CD23− lymphocyte (**b**) numbers plotted (cordocentesis □, caesarean section ○) as a function of length of gestation (CD19+CD23+ number: $p < 0.0001$, $r = 0.403$, CD19+CD23− number: median = 0.26×10^9/l, 95% confidence interval = $0.04–0.62 \times 10^9$/l; n = 176). The sloping lines are the mean, 2.5th and 97.5th percentile values.

Table 3. Reference ranges for the number of fetal CD19+CD10− and CD19+CD23+ and lymphocytes (mean and 95% confidence interval) with gestation (GA)

GA weeks	CD19+CD10−, $\times 10^9$/l		CD19+CD23+, $\times 10^9$/l	
	mean	95% CI	mean	95% CI
16	0.07	0.00–0.28	0.11	0.00–0.39
18	0.08	0.00–0.30	0.12	0.00–0.41
20	0.09	0.00–0.33	0.14	0.01–0.45
22	0.11	0.00–0.36	0.15	0.02–0.49
24	0.13	0.00–0.39	0.17	0.02–0.53
26	0.15	0.00–0.43	0.19	0.03–0.58
28	0.17	0.01–0.47	0.21	0.04–0.63
30	0.20	0.02–0.51	0.23	0.05–0.68
32	0.22	0.03–0.56	0.26	0.06–0.74
34	0.25	0.05–0.61	0.28	0.07–0.81
36	0.27	0.06–0.66	0.31	0.08–0.88
38	0.30	0.08–0.72	0.34	0.09–0.95
40	0.33	0.09–0.78	0.37	0.10–1.03

Discussion

The findings of this study demonstrate that although there is an exponential increase in the total number of B lymphocytes up to 28 weeks gestation, maturation of B cells, as evidenced by changes in expression of CD5, CD10 and CD23, occurs mainly in the third trimester.

The CD5+ antigen characterizes a minor subpopulation of B lymphocytes in adults and older children, which produce low-affinity, polyreactive antibodies that cross-react with autoantigens [10]. The number of CD5+ B cells is elevated in auto-immune conditions such as rheumatoid arthritis [11]. In contrast to antibody production in mature B cells, fetal CD5+ B lymphocytes express germ-line encoded antibody idiotypes which are unmodified by somatic mutation [12]. The finding that the majority of cells produced by the fetus from at least 17 weeks gestation are of this immature phenotype appears to be an important characteristics of fetal B-lymphocyte development.

The CD10 and CD19 antigens are co-expressed on immature Pre-B cells at a very early maturational stage in human bone marrow [13]. As B lymphocytes mature, specificity for antigen is acquired by immunoglobulin gene rearrangements and the CD10 antigen is lost [14]. The finding of this study that the proportion of B cells expressing the immature CD10 antigen falls suddenly after 28 weeks suggests that increasing antigen specificity occurs after this period.

The CD23 antigen is expressed by activated B lymphocytes, and an increase in its expression may be due to continuous antigenic exposure [15]. The increased expression of CD23+ with gestation demonstrated in this study may have identified an endogenously activated B-cell population. Major histocompatibility complex class II antigens (HLA-DR) are required on the cell surface to allow B-T lymphocyte co-operation and evolution of antibody production. The finding that all B lymphocytes from at least 17 weeks gestation express HLA-DR is consistent with current theories on the ontogeny of B cells.

The changes in CD5, CD10 and CD23 expression that reflect fetal B lymphocyte maturation occur later in pregnancy relative to the maturation of fetal T lymphocytes [16]. These changes may be controlled by a genetically determined developmental clock or may be the result of changing fetal physiological priorities during gestation. Thus, the placenta acts as an effective barrier to most bacteria [17] and therefore, the acquisition of humoral defence mechanisms is only necessary later on in pregnancy, in preparation for extra-uterine life. This assumption would be consistent with the finding that the number of circulating fetal neutrophils also increases exponentially during the late third trimester [1]. This study has established reference ranges with gestation for various fetal B lymphocyte subpopulations, demonstrating that the majority of B cell maturation occurs late in the third trimester. This finding may be one factor that contributes to the vulnerability of premature neonates to bacterial infection [18], which is a significant cause of perinatal mortality and morbidity [19]. A clearer understanding of the mechanisms controlling lymphocyte maturation in the fetus could provide the basis for therapeutic manipulation aimed at the prevention of infection in these neonates.

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