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Fetal Blood Mononuclear Cell Division in Normal and Pathological Pregnancies

Abstract

Fetal blood mononuclear cell division was measured using flow cytometry in 53 normal pregnancies and 51 pathological pregnancies complicated either by anaemia due to red blood cell isoimmunisation (RCI: n = 21), intrauterine growth retardation (SGA: n = 13) or abnormal karyotype (n = 17). In normal pregnancy, mononuclear cell division rates decreased with gestational age from a mean of 1.8% at 18 weeks to 1% at 40 weeks. Furthermore, there was a significant association between cell division and erythroblast count. The rates of cell division and erythroblast count were significantly increased in the chromosomally abnormal fetuses, and significantly decreased in the transfused RCI fetuses compared to the controls. Although the erythroblast count was elevated in the SGA fetuses, the mononuclear cell division was not significantly different from the controls. Fetal blood mononuclear cell division is elevated in early pregnancy and in chromosomally abnormal fetuses, probably as a consequence of increased numbers of circulating haemopoietic precursors. Mononuclear cell division is decreased in transfused RCI fetuses as a consequence of suppressed erythropoiesis. In SGA fetuses, despite the increased erythropoietic stimulation and erythroblastosis, cell division is not increased.

Key Words
Fetal blood
Cordocentesis
Cell division
Aneuploidy
Hypoxia
Anaemia

Introduction

In postnatal life, blood mononuclear cells undergoing DNA replication or cellular division are mainly confined to the marrow as haemopoietic progenitors [1], and in the absence of malignancy more than 99% of peripheral blood mononuclear cells (mainly leucocytes) are in the resting phase of the cell cycle [2]. In fetal life, there is a high number of

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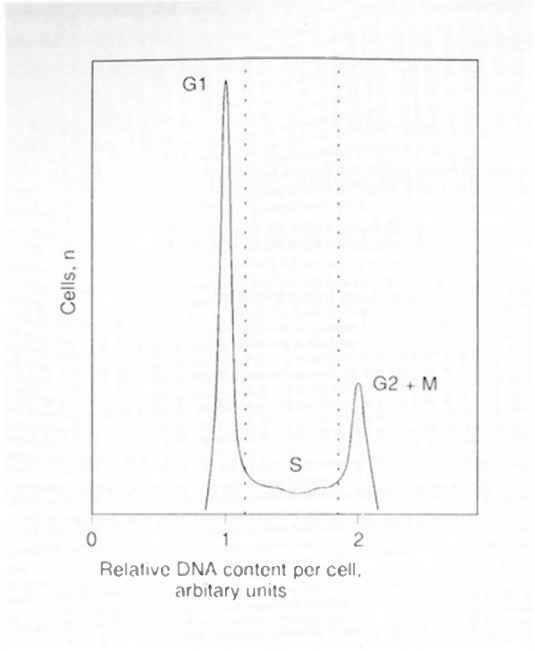

Fig. 1. Relative DNA content per cell (fluorescence intensity) demonstrating non-dividing (G1 phase) and dividing (S, G2 and M phases) cells.

circulating haemopoietic stem cells probably as a consequence of the increased activity of growth factors and the inability of the liver to prevent the release of nucleated precursors [3, 4]. The aim of this study is to investigate fetal blood mononuclear cell (haemopoietic stem cells, erythroblasts and leucocytes) division and its association with gestational age and the number of circulating haemopoietic precursors, both in normal and pathological pregnancies.

Patients and Methods

Mononuclear cell division and erythroblast counts were determined in fetal blood samples obtained by (1) fetal cardiocentesis immediately before elective termination of pregnancy for social indications at 13–17 weeks (n = 12); (2) umbilical cord puncture at delivery, from elective caesarean sections at 38–40 weeks for previous section or breech presentation (n = 12), and (3) cordocentesis for fetal karyotyping at 18–37 weeks for minor malformations such as mild hydronephrosis and choroid plexus cysts where the fetal karyotype was subsequently found to be normal (n = 29). In addition, blood was obtained by cordocentesis from (1) 21 red blood cell isoimmunised (RCI) pregnancies, which included 12 fetuses that had been transfused on previous occasions and 9 that were being sampled for the first time; (2) 13 pregnancies with ultrasound evidence of severe fetal growth retardation (SGA), where the fetal karyotype was subsequently found to be normal, and (3) 17 pregnancies with chromosomally abnormal fetuses including trisomy 21 (n = 5), trisomy 18 (n = 3), trisomy 13 (n = 3), Turner syndrome (n = 4), and triploidy (n = 2). The study was cross-sectional and in each case, gestational age was determined from the menstrual history and confirmed by an ultrasound scan in early pregnancy. Kleihauer-Betke testing confirmed that all blood samples contained only fetal cells (except in the transfused RCI pregnancies). Blood films were stained by the May-Grünwald-Giemsa method for the differential cell count. Fetal blood (0.5 ml) was collected into isotonic edetic acid solution (0.5 mmol/l in 0.15 mmol/l sodium chloride) and single-cell suspension of nucleated cells was prepared by density gradient centrifugation [5]. The DNA stained with propidium iodide (Sigma Chemicals, Poole, England), and cytometric analysis was carried out using a FACScan and Cellfit software (Becton Dickinson, Oxford, England). A minimum of 10,000 cells were acquired to calculate the percentage of cells in the various stages of cell division (fig. 1).

Statistics

Regression analysis was used to determine the significance of any association between the cell division rate and both erythroblast count and gestational age. Since in normal pregnancy the cell division rate and the erythroblast count change with gestation [6], the fetal values obtained from the pathological pregnancies were expressed as the number of standard deviations (SD) by which the individual values different from the appropriate normal mean for gestation (delta values). Unpaired Student's t test was applied to determine if the mean delta values for cell division and erythroblastosis in the RCI, SGA and chromosomally abnormal fetuses differed significantly from the appropriate normal mean for gestation.

Results

In the fetal blood samples obtained before 16 weeks gestation, both the rate of cell division (fig. 2: median = 3.2%, range = 0.7–14.7) and erythroblast count (median = 14.7 × 10^9/l, range = 2.5–23.9 × 10^9/l) were significantly higher than in those obtained at later gestations (cell division: median = 1.4%, t = 2.60, $p < 0.001$; erythroblast count: median = 0.5 × 10^9/l, t = 4.33, $p < 0.0001$). For the samples obtained after 16 weeks gestation, there was a linear decrease in the percentage of dividing mononuclear cells from a mean of 1.8% at 18 weeks to a mean of 1.0% at 40 weeks (fig. 2: $r = 0.403$, $p < 0.001$). Furthermore, there was a significant association between the rate of cell division and the number of erythroblasts (fig. 2: $r = 0.409$, $p < 0.05$). In the abnormal karyotype group, both the mean rate of cell division and the erythroblast count were significantly higher than in the controls (fig. 3: cell division: t = 4.07, $p < 0.001$; erythroblast count: t = 12.33, $p < 0.0001$). The highest rates were observed in two trisomy 21 fetuses who also had markedly elevated leucocyte counts of 9.7 and 19.5 × 10^9/l; in all other cases the leucocyte count was less than 4.0 × 10^9/l. In the untransfused RCI fetuses (haemoglobin concentration: median = 10.8 g/dl, range = 5.4–12.0 g/dl), both the mean rate of cell division and the erythroblast count were not significantly different from the controls. However, in the RCI post-transfusion group, both cell division and erythroblast count were significantly decreased (fig. 3: cell division: t = −2.62, $p < 0.05$; erythroblast count: t = −3.60, $p < 0.01$). In the SGA fetuses, the erythroblast count was significantly increased (fig. 3: t = 5.70, $p < 0.0001$), but the mean rate of cell division and haemoglobin concentration (median = 12.3 g/dl, range = 10.5–18.3 g/dl) was not significantly different from the controls (cell division: t = 0.14, haemoglobin concentration: t = 1.24).

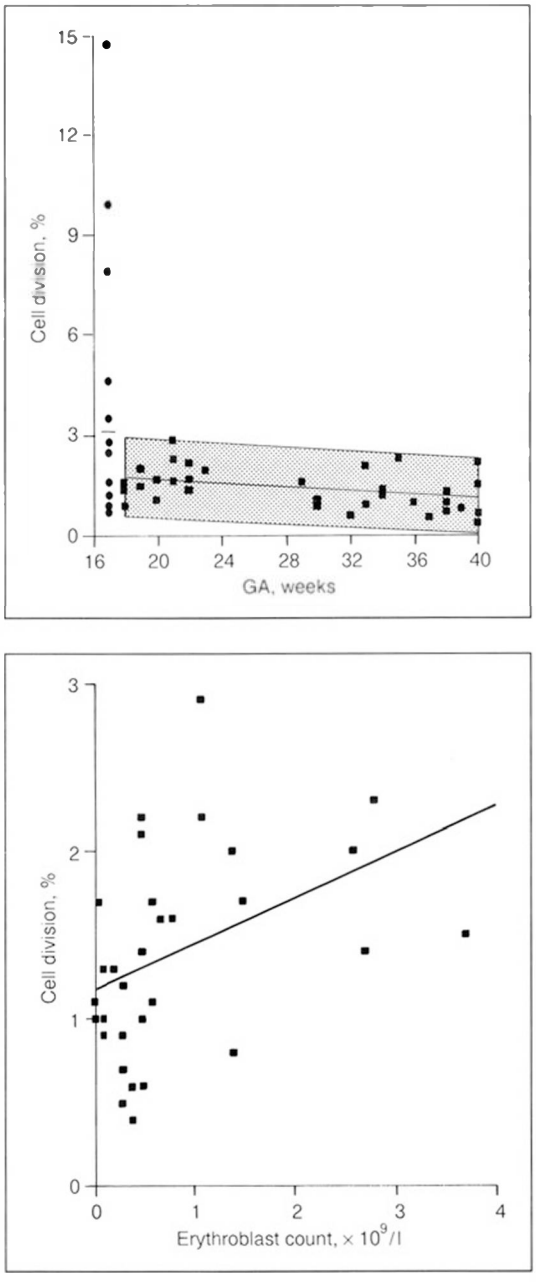

Fig. 2. Fetal blood mononuclear cell division plotted as a function of length of gestation (**a**). The sloping lines are the mean, 2.5th and 97.5th percentile values. The individual values on the left are for samples at 13–17 weeks gestation, and the horizontal bar is the median. Relationship of fetal blood mononuclear cell division with erythroblast count (**b**).

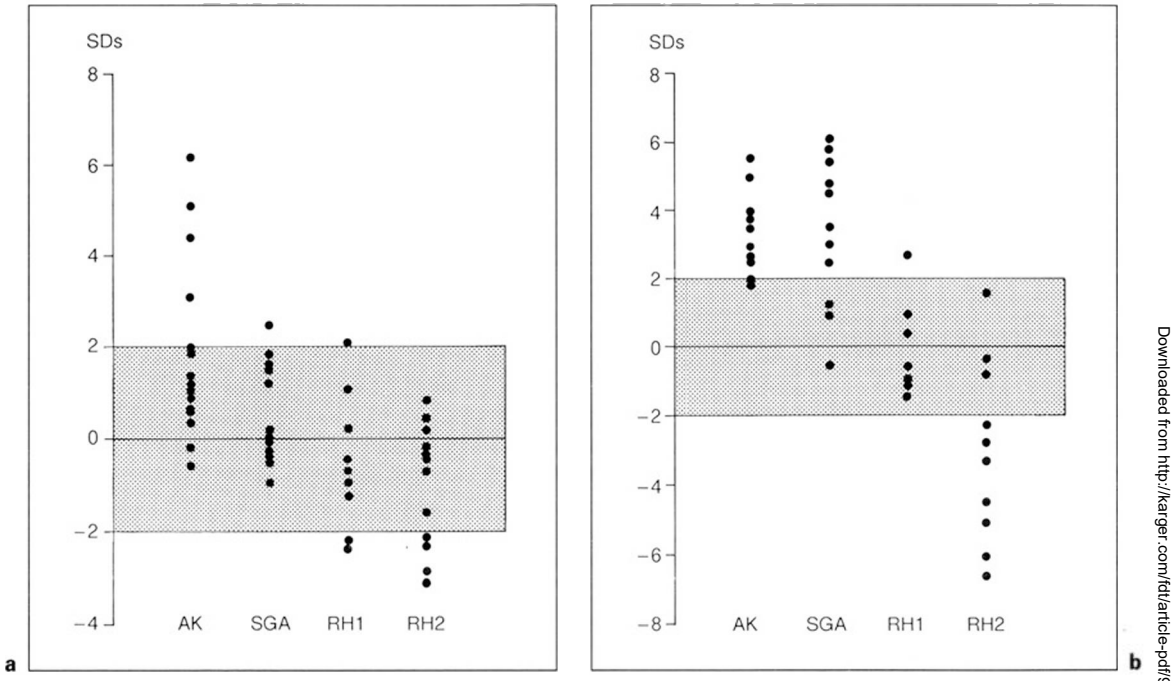

Fig. 3. Individual values of fetal blood mononuclear cell division (**a**) and erythroblast count (**b**) plotted as standard deviations (SDs) from the normal mean for gestation for pregnancies with chromosomally abnormal (AK) and growth retarded (SGA) fetuses, and in red blood cell isoimmunised pregnancies before (RH1) and subsequent to intrauterine transfusions (RH2).

Discussion

The findings of this study demonstrate that fetal blood mononuclear cell division is higher in early than late pregnancy and is associated with blood erythroblast count. The high numbers of rapidly dividing, circulating haemopoietic cells may be the consequence of increased levels and activity of growth factors. At this stage of fetal life, growth factors and cytokine levels are elevated, presumably due to production in the decidual or feto-placental unit [7, 8]. Additionally, fetal tissues have been shown to be more sensitive than adult tissues to these factors [3]. Alternatively, these findings may be the consequence of extramedullary erythropoiesis. Thus, in early pregnancy the liver is the major erythropoietic organ, which unlike the marrow is thought to allow the escape of nucleated haemopoietic precursors such as erythroblasts and haemopoietic stem cells into the circulation [4, 9, 10].

In the SGA group, there was erythroblastosis, which is thought to be the consequence of tissue hypoxia and erythropoietin mediated stimulation of extramedullary erythropoiesis [11]. However, the rates of cell division and haemoglobin concentration were not significantly different from the controls. It is possible that despite the apparent stimulation of erythropoiesis and release of erythroblasts, there is a hypoxia-induced suppression of cell

division and therefore failure to increase the haemoglobin concentration [12, 13].

In red blood cell isoimmunised pregnancies, extramedullary erythropoiesis and erythroblastosis are known to occur when the fetal haemoglobin deficit is more than 7 g/dl [4, 9]. In this study, none of the pre-transfused fetuses were severely anaemic, and therefore both cell division and erythroblast count were not siginificantly different from controls. However, in fetuses that had received transfusions, erythropoiesis is suppressed and consequently both the percentage of dividing cells and the number of erythroblasts were decreased [14].

In chromosomally abnormal fetuses, both the percentage of dividing cells and the number of erythroblasts were increased. As these variables are known to decrease exponentially with gestational age, these findings may be the consequence of the 'developmental delay' that is characteristic of fetuses with an abnormal karyotype [15]. Alternatively, the increased numbers of rapidly dividing, circulating haemopoietic cells may be the consequence of abnormal regulation of haemopoiesis in these fetuses. The latter hypothesis is supported by the finding in this study that the 2 trisomy 21 fetuses with striking erythroblastosis and leucocytosis had the highest cell division rates, and the finding in post-natal life that chromosomally abnormal fetuses are more susceptible to malignancies of haemopoietic origin [16, 17].

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