British Journal of Obstetrics and Gynaecology
April 1994, Vol. 101, pp. 315–318

FETAL AND NEONATAL MEDICINE

Fetal lymphocyte subpopulations in red blood cell iso-immunised pregnancies

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ABSTRACT

Objective To study the association between fetal anaemia and alterations in lymphocyte subpopulations.

Design Cross-sectional study.

Setting The Harris Birthright Research Centre for Fetal Medicine, King's College Hospital Medical School, London.

Subjects Forty-three red blood cell iso-immunised pregnancies undergoing cordocentesis at 19 to 38 weeks gestation.

Main outcome measures Fetal blood haemoglobin concentration, erythroblast count and lymphocyte subpopulations.

Results The mean T (CD3+), B (CD19+), T-helper (CD4+), T-suppressor/cytotoxic (CD8+) and natural killer (NK: CD16+/CD56+) cell counts in the anaemic fetuses were significantly lower than the appropriate normal mean for gestation (CD3+: $t = -3{\cdot}25$, $P < 0{\cdot}01$; CD19+: $t = -2{\cdot}14$, $P < 0{\cdot}05$; CD4+: $t - 4{\cdot}03$, $P < 0{\cdot}001$; CD8+: $t = -3{\cdot}39$, $P < 0{\cdot}01$ and CD16+/CD56+: $t = -3{\cdot}49$, $P < 0{\cdot}01$). Furthermore, there was a significant association between the decrease in T lymphocyte number and the degree of fetal anaemia ($r = 0{\cdot}342$, $P < 0{\cdot}05$).

Conclusions Fetuses from red blood cell iso-immunised pregnancies exhibit nonselective lymphopenia that is proportional to the degree of anaemia.

Fetal tissue hypoxia, either due to impaired placental perfusion and oxygenation or due to fetal anaemia, as seen in red blood cell iso-immunisation, is associated with increased fetal plasma erythropoietin concentration (Snijders *et al.* 1993; Thilaganathan *et al.* 1993a). In both conditions, the increased erythropoietin level is associated with fetal erythroblastosis, thrombocytopenia and leucopenia, and it was suggested that these findings may represent channelling of fetal haemopoiesis in favour of erythropoiesis and at the expense of the other lineages (Migliaccio & Migliaccio 1988; Koenig & Christensen 1989; Davies *et al.* 1991, 1992). However, study of fetal lymphocyte subpopulations in intrauterine growth retardation has demonstrated that there is selective suppression of T-cells and T-helper cells, whereas T-suppressor cells, B-cells and natural killer (NK) cells are unaffected (Thilaganathan *et al.* 1993b). The aim of this study was to investigate if anaemic hypoxia is also associated with selective suppression of certain lymphocyte subpopulations or whether the lymphopenia is nonspecific.

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Subjects and methods

During the period August 1991 to September 1992, fetal lymphocyte subpopulations were measured in umbilical venous blood samples obtained by cordocentesis at 19 to 34 weeks gestation from 43 consecutive red blood cell iso-immunised pregnancies that had not received previous intrauterine blood transfusions. Kleihauer-Betke testing confirmed that all blood samples contained only fetal blood. Blood samples (180 µl) were collected into 20 µl of isotonic edetic acid solution (0·5 mmol/l in 0·15 mmol/l NaCl) for determination of the full blood count (Coulter S-Plus, Coulter Electronics, Luton, UK) and differential cell count (May-Grünwald-Giemsa stain). Blood samples (500 µl) were also collected into heparin for enumeration of lymphocyte subpopulations.

Simultaneous two-colour determination of lymphocyte

subsets in whole blood was carried out using a FACScan (Caldwell & Taylor 1986), Consort 32 software and monoclonal antibodies: CD45/CD14, CD3/CD19, CD4/CD8 and CD3/CD16 & CD56 (Becton Dickinson UK Ltd, Oxford, UK). Samples were gated using forward angle and 90° light-scattering properties to exclude granulocytes, monocytes and platelets. Control staining of fetal cells with anti-mouse monoclonal IgG_{2a}-PE/IgG_1-FITC was performed on each sample, and background readings of less than 1% were obtained. A minimum of 5000 cells were acquired in the lymphocyte fraction and analysed to calculate the percentage of each subset. The absolute number of cells was derived from the total nucleated cell count, the lymphocyte differential count on the blood film and the percentage of lymphocytes.

Since in normal pregnancy the fetal haemoglobin concentration, erythroblast count and lymphocyte subpopulations change with gestation (Nicolaides *et al.* 1988, 1989; Thilaganathan *et al.* 1992, 1993c, d), the values obtained from the red blood cell iso-immunised fetuses were expressed as the number of standard deviations (SD) by which the individual values differed from the normal mean for gestation (delta value or SD score). The one sample *t*-test was used to determine if the values in the anaemic fetuses were significantly different from the normal mean for gestation. Regression analysis was used to determine the significance of any association between delta haemoglobin concentration and delta values for other parameters.

Results

In the red blood cell iso-immunised pregnancies, the mean fetal haemoglobin concentration was significantly lower and the erythroblast count was significantly higher than the normal mean for gestation. As shown in Fig. 1, haemoglobin concentration: mean difference $= -3{\cdot}263$, SEM $= 0{\cdot}458$, $t = -7{\cdot}13$, $P < 0{\cdot}0001$; erythroblast count: mean difference $= -0{\cdot}517$, SEM $= 0{\cdot}231$, $t = -2{\cdot}24$, $P < 0{\cdot}05$). Furthermore, the mean T (CD3+), B (CD19+), T-helper (CD4+), T-suppressor/cytotoxic (CD8+) and the NK (CD16+/CD56+) cell counts were significantly lower than the appropriate normal mean for gestation. As

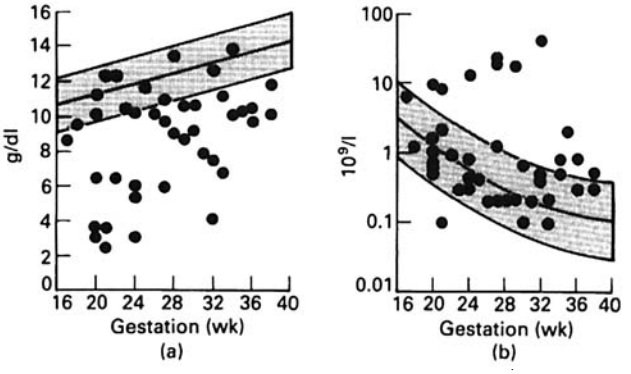


Fig. 1. Haemoglobin concentration (a) and erythroblast count (b) in the 43 fetuses from red blood cell iso-immunised pregnancies plotted on the appropriate reference range (mean, 5th and 95th centiles) for gestation.

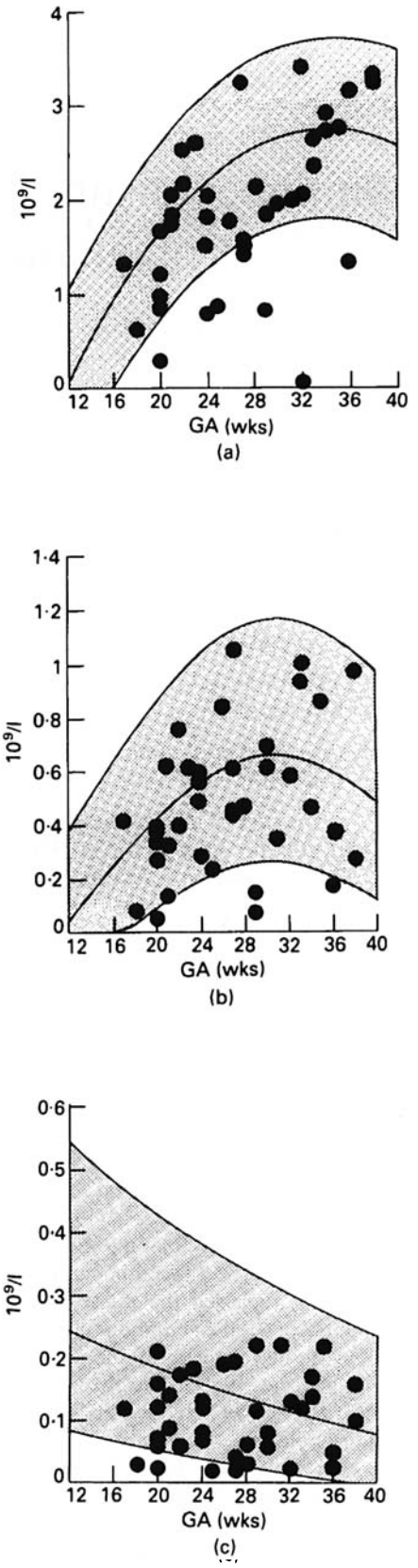
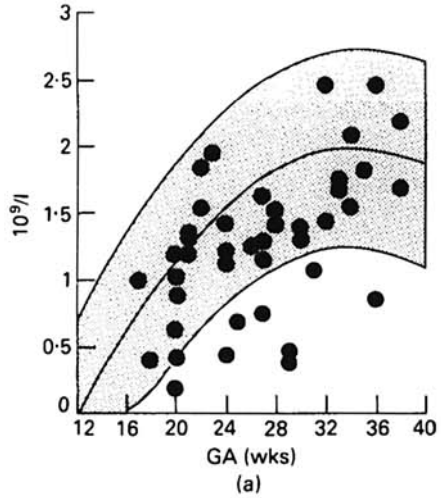
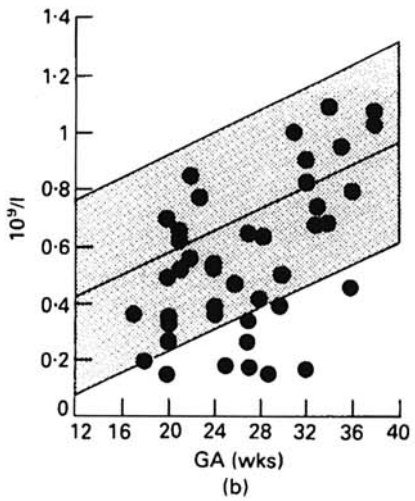


Fig. 2. The T (CD3+) (a), B (CD19+) (b) and NK (CD16+/CD56+) (c) cell counts in the 43 fetuses from red blood cell iso-immunised pregnancies plotted on the appropriate reference range (mean, 5th and 95th centiles) for gestation.





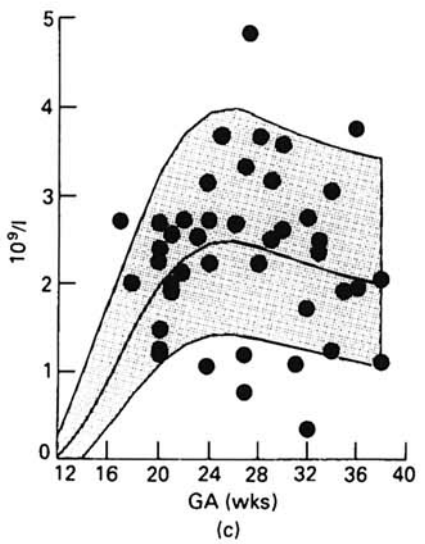


Fig. 3. The T-helper (CD4+) (a), T-suppressor/cytotoxic (CD8+) (b) and CD4 to CD8 ratio (c) in the 43 fetuses from red blood cell iso-immunised pregnancies plotted on the appropriate reference range (mean, 5th and 95th centiles) for gestation.

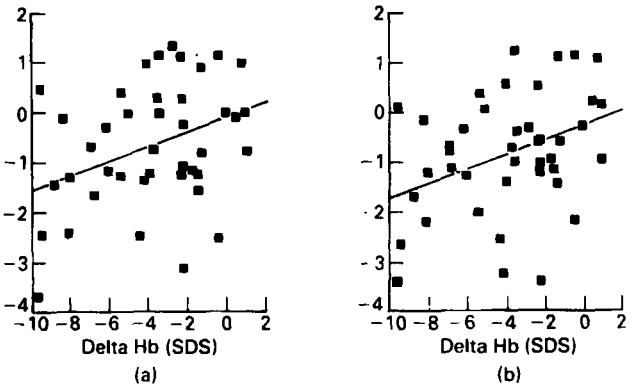


Fig. 4. Relation of delta haemoglobin concentration with delta CD3+ count (a) and delta CD4+ count (b) in the 43 fetuses from red blood cell iso-immunised pregnancies. Delta values are the number of standard deviations or standard deviation score (SDS) by which the individual values differed from the appropriate normal mean for gestation. The most lymphopenic fetuses were also anaemic.

shown in Figs 2 and 3, CD3+: mean difference = −0·637, SEM = 0·196, $t = -3·25$, $P < 0·01$; CD19+: mean difference = −0·112, SEM = 0·053, $t = -2·14$, $P < 0·05$; CD4+: mean difference = −0·768, SEM = 0·191, $t = -4·03$, $P < 0·001$; CD8+: mean difference = −0·688, SEM = 0·203, $t = -3·39$, $P < 0·01$ and CD16+/CD56+: mean difference = −0·812, SEM = 0·232, $t = -3·49$, $P < 0·01$), but the mean CD4+ to CD8+ ratio was not significantly different (Fig. 3: mean difference = 0·011, SEM = 0·030, $t = 0·38$). There were significant associations between delta haemoglobin concentration and delta CD3+ and delta CD4+. As shown in Fig. 4, delta CD3+: $r = 0·342$, $P < 0·05$; delta CD4+: $r = 0·348$, $P < 0·05$).

Discussion

The finding that in red blood cell iso-immunisation anaemia is associated with nonspecific decrease in all the circulating fetal lymphocyte subpopulations suggests that in fetal anaemia the compensatory increase in erythropoiesis is at the expense of lymphopoiesis. This hypothesis is supported by the finding that in newborns with severe red blood cell iso-immunisation, the increase in the number of bone marrow erythroid progenitors is at the expense of other lineages (Migliaccio & Migliaccio 1988; Koenig & Christensen 1989; Millard *et al.* 1990). An alternative explanation for the observed T, B and NK cell lymphopenia is nonspecific haemophagocytosis due to activation of the reticulo–endothelial system which has been observed in autoimmune haemolytic anaemia in adults (Suster *et al.* 1988).

Unlike red blood cell iso-immunisation, in intrauterine growth retardation due to impaired placental perfusion, the associated lymphopenia selectively affects the T (CD3+) and T-helper cell (CD4+) lineages (Thilaganathan *et al.* 1993b). The most likely explanation for this difference is that in fetal anaemia, placental perfusion and nutrition are normal; in contrast, in fetal growth

retardation impaired placental perfusion leads to a nutritionally deprived state (Economides *et al.* 1989, 1990). This hypothesis is supported by the finding that selective lymphopenia affecting mainly T and T-helper cell subpopulations is also found in postnatal malnutrition (Chandra 1983). Irrespective of the underlying cause, the decreased T and B cell numbers and consequent impairment of fetal cell-mediated and humoral immune responses in red blood cell iso-immunised pregnancies, may explain the predisposition to infections in these infants (Panagopoulos *et al.* 1969; Berkowitz 1991).

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Received 18 May 1993
Accepted 6 September 1993