

Fetal leucocyte count in rhesus disease

N P Davies, A G S Buggins, R J M Snijders, P N Noble, D M Layton, K H Nicolaides

Abstract

The effect of fetal anaemia on the total and differential leucocyte counts was studied by examining blood samples obtained by cordocentesis from 177 previously untransfused rhesus affected fetuses at 17-36 weeks' gestation. The mean fetal total leucocyte, lymphocyte, and monocyte counts were significantly lower than the corresponding values in normal controls and there were significant associations between the decrease in these cells and the degree of fetal anaemia. Possible mechanisms for leucopenia include (i) stimulation of erythroid progenitor production at the expense of production of myeloid progenitors, (ii) non-specific haemophagocytosis, or (iii) general suppression of haemopoiesis. Further understanding of the underlying mechanism and the implications of leucopenia as well as the previously reported thrombocytopenia and anaemia may provide a basis for improved antenatal and/or postnatal treatment.

In red cell isoimmunised pregnancies the life span of fetal erythrocytes is reduced because antibody coated red cells are destroyed in the fetal reticuloendothelial system. In mild-moderate anaemia there is associated reticulocytosis suggesting a compensatory increase in intramedullary erythropoiesis.¹ With severe anaemia there is recruitment of extramedullary

erythropoietic sites resulting in macrocytosis and erythroblastaemia.^{1,2} In mild-moderate anaemia there is associated thrombocytosis and in severe anaemia there is thrombocytopenia.³

The aim of the present study was to examine the effect of anaemia due to rhesus isoimmunisation on the total and differential leucocyte counts.

Patients and methods

The total and differential fetal leucocyte counts were determined in a cross sectional study of 177 previously untransfused fetuses from rhesus isoimmunised pregnancies at 17-36 weeks' gestation.

Gestation was determined from the menstrual history or an ultrasound scan performed in early pregnancy. In 32 cases ultrasound evidence of fetal hydrops (skin oedema, ascites, pleural or pericardial effusions) was found. Umbilical cord blood samples were obtained by cordocentesis, which was performed without maternal sedation or fetal paralysis.⁴

Fetal blood (180 μ l) was collected into 20 μ l of isotonic edetic acid solution (0.5 mmol/l in 0.15 mmol/l of sodium chloride). The full blood count was determined on a Coulter Stacker Automated Cytometer (Coulter Electronics). Blood films were stained with May-Grünwald-Giemsa on an automatic processing machine and the nucleated red blood cell (NRBC) count

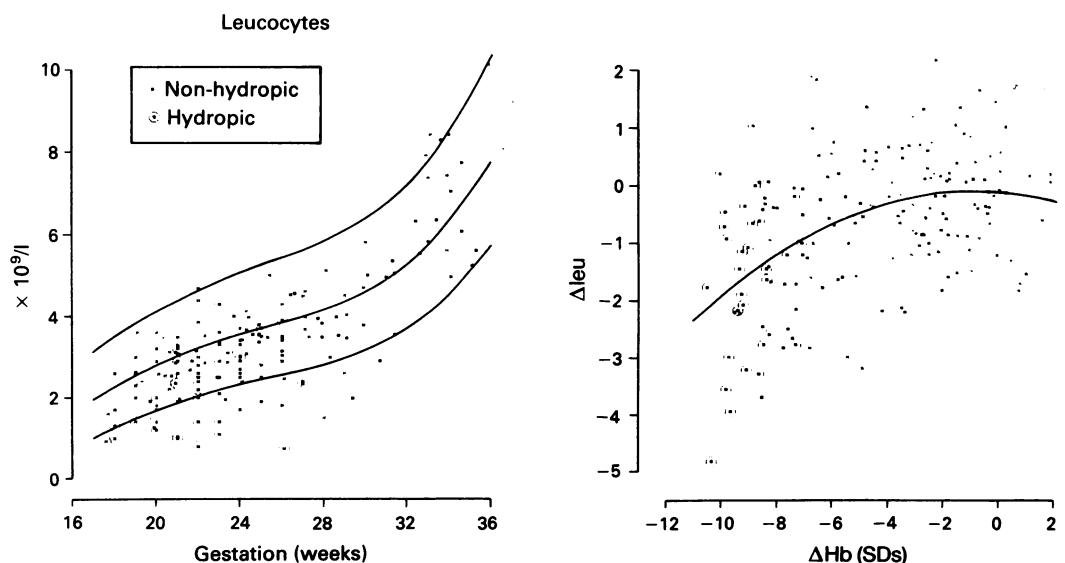


Figure 1 Total fetal blood leucocyte count in 145 non-hydropsic and 32 hydropsic fetuses from red cell isoimmunised pregnancies plotted on the reference range (mean, 5th, and 95th centiles) for gestation. There is a significant association between the degree of fetal leucopenia (Δ leu) and anaemia (Δ Hb); Δ values represent differences from the appropriate normal mean for gestation in SDs.

King's College School
of Medicine and
Dentistry, London,
Harris Birthright
Research Centre
for Fetal Medicine
N P Davies
R J M Snijders
P N Noble
K H Nicolaides
Department of
Haematological
Medicine
A G S Buggins
D M Layton

Correspondence to:
Dr K H Nicolaides,
Harris Birthright
Research Centre
for Fetal Medicine,
Department of Obstetrics
and Gynaecology,
King's College School
of Medicine and
Dentistry, Denmark Hill,
London SE5 8RX.

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per 100 leucocytes was determined. The corrected leucocyte count was calculated (leucocyte count=uncorrected leucocyte count \times 100/NRBC count+100). To determine the differential count, 100 leucocytes were examined and classified. Kleihauer testing confirmed that all samples were fetal. Furthermore, in all cases used for this study the Coombs' test was positive.

In normal pregnancy the fetal haemoglobin concentration, platelet, and total and differential leucocyte counts change with gestation.^{3 5 6} The values obtained from the rhesus isoimmunised pregnancies were expressed as the number of standard deviations (SDs) by which the individual values differed from the normal mean for gestation (Δ value). Two tailed

Student's *t* test was applied to determine if there were significant differences between the findings in rhesus affected pregnancies and normals. Regression analysis was applied to determine the significance of any associations between Δ values for the individual parameters.

Results

In the rhesus affected pregnancies the mean fetal total leucocyte, lymphocyte, and monocyte counts were significantly lower than the corresponding values in normal controls.⁶ This is shown in figs 1-3; mean (SEM) difference=-0.637 (0.09) SDs, *t*=-6.81, *p*<0.0001; mean difference=-0.687 (0.08) SDs, *t*=-8.3, *p*<0.0001; and mean difference=-0.536 (0.08) SDs, *t*=-6.77, *p*<0.0001 respectively. There

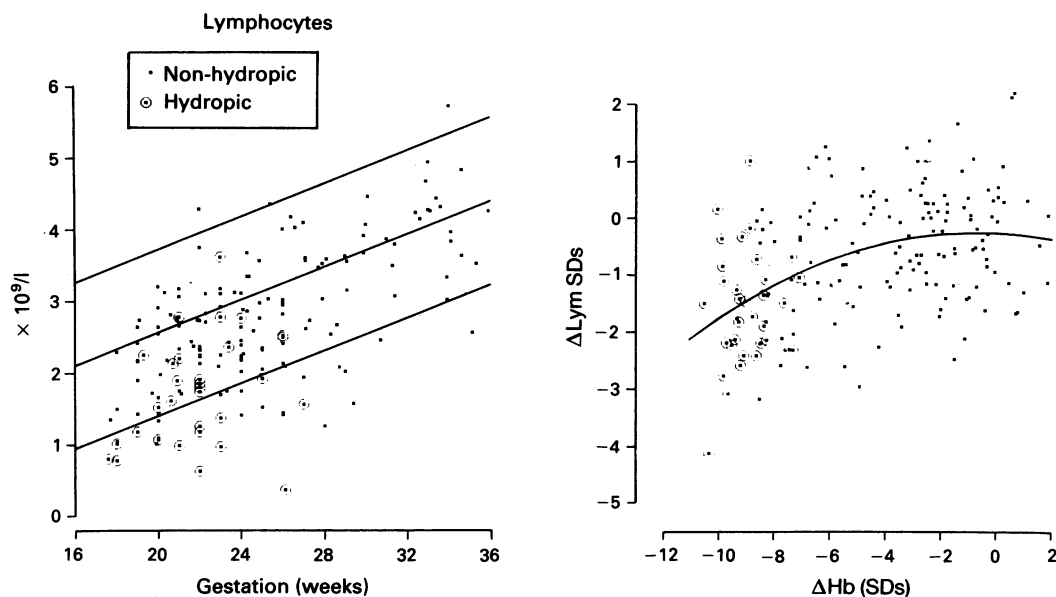


Figure 2 Fetal blood lymphocyte count in 145 non-hydronic and 32 hydronic fetuses from red cell isoimmunised pregnancies plotted on the reference range (mean, 5th, and 95th centiles) for gestation. There is a significant association between the degree of fetal lymphocytopenia (Δ Lym) and anaemia (Δ Hb); Δ values represent differences from the appropriate normal mean for gestation in SDs.

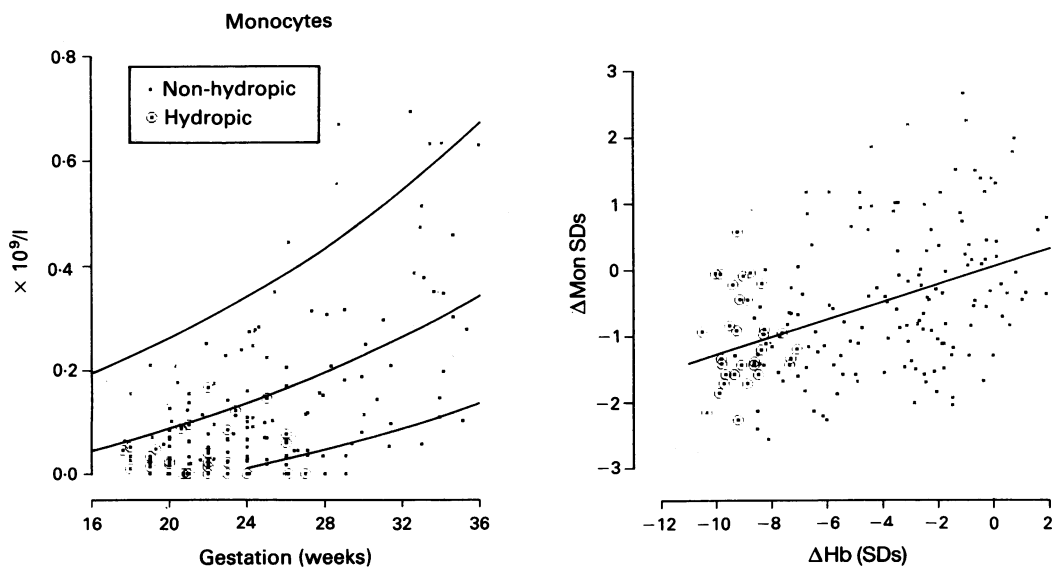


Figure 3 Fetal blood monocyte count in 145 non-hydronic and 32 hydronic fetuses from red cell isoimmunised pregnancies plotted on the reference range (mean, 5th, and 95th centiles) for gestation. There is a significant association between the degree of fetal monocytopenia (Δ Mon) and anaemia (Δ Hb); Δ values represent differences from the appropriate normal mean for gestation in SDs.

were no significant differences in mean neutrophil (mean difference=0.112 (0.09) SDs, $t=1.19$) or eosinophil counts (mean difference=0.025 (0.02) $\times 10^9/l$, $t=1.68$).

There was a significant association between Δ total leucocyte count and Δ haemoglobin concentration which was best described by a quadratic equation (fig 1; $r=0.470$, $p<0.0001$). Multiple regression analysis showed that hydrops did not have a significant independent contribution to this association (F to remove hydrops=2.93, $p=0.09$). Similarly, there was a significant quadratic association between Δ lymphocyte count and Δ haemoglobin concentration (fig 2; $r=0.439$, $p<0.05$). There was a significant linear association between Δ monocyte count and Δ haemoglobin concentration (fig 3; $r=0.413$, $p<0.0001$).

Discussion

In rhesus isoimmunisation fetal haemolysis is associated with leucopenia. Lymphocyte and monocyte counts decreased in proportion to the degree of fetal anaemia, although neutrophil numbers appeared to be unaffected. Koenig and Christensen, using blood samples obtained postnatally from infants of rhesus affected pregnancies, also reported an association between leucopenia and severe anaemia.⁷ However, their findings suggested that the infants were neutropenic. A possible explanation for this apparent discrepancy is that in intrauterine life, especially before 32 weeks' gestation, neutrophil counts are normally quite low (1–3 per 100 white cells) making it difficult to demonstrate a significant reduction.⁶

Haemopoiesis defines the production of functionally distinct mature blood cells from a common pluripotent progenitor. It is possible that in severe anaemia due to rhesus isoimmunisation the observed leucopenia, as well as the previously described thrombocytopenia,³ may reflect the coordinated differentiation of erythroid progenitors at the expense of myeloid and megakaryocytic maturation. Although such 'channelling' of haemopoietic reserve is not commonly observed in postnatal haemolysis, Koenig and Christensen have reported that in some neonates with severe red cell isoimmunisation there is an increase in the number of erythroid progenitors accompanied by a reduction in numbers of granulocyte-macrophage progenitors.⁷ Furthermore, addition of erythropoietin to cultures of human fetal progenitors reduces the number of granulocyte-macrophage colonies formed in response to granulocyte-macrophage colony stimulating factor.⁸

A further possible explanation for leucopenia and thrombocytopenia in red cell isoimmunisation is non-specific haemophagocytosis due to activation of the reticuloendothelial system.⁹

This process has been observed in autoimmune haemolytic anaemia.¹⁰ Although the phenomenon was thought to be rare, Suster *et al* have recently examined 230 consecutive adult necropsies and found evidence of moderate to severe haemophagocytosis in the bone marrow of 102 cases.¹¹

In those cases where leucopenia and thrombocytopenia are associated with absence of an erythroid response, marrow suppression due to tissue hypoxia and lactic acidosis may be important.^{12 13} Additionally, as in postnatal life, severe haemolysis and consequent increased erythropoietic activity could result in relative deficiency of haematinics such as folate.¹⁴

In summary, this study demonstrates that in rhesus affected fetuses severe anaemia is not only accompanied by thrombocytopenia but also by leucopenia. Possible mechanisms causing the reduced numbers of white cells and platelets include (i) stimulation of erythroid progenitor production at the expense of production of myeloid and megakaryocyte progenitors, (ii) non-specific haemophagocytosis, or (iii) general suppression of haemopoiesis. Further studies on the underlying mechanism and the implications of presence of leucopenia as well as thrombocytopenia and anaemia may provide a basis for improved antenatal and/or postnatal treatment.

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