Fetal immunodeficiency: a consequence of placental insufficiency

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ABSTRACT

Objective To study the effect of placental insufficiency on fetal lymphocyte subpopulations.

Study design Cross sectional study of 19 growth retarded fetuses undergoing cordocentesis at 24 to 37 weeks gestation. Flow cytometry was used to enumerate fetal blood lymphocyte subpopulations.

Results The mean T (CD3+), B (CD19+), T-helper (CD4+), T-suppressor/cytotoxic (CD8+) cell counts and the CD4 to CD8 ratio in the growth retarded fetuses were significantly lower than the respective normal mean for gestation (CD3+: z = 3·66, $P<0·001$; CD19+: z = 2·18, $P<0·05$; CD4+: z = 3·76, $P<0·001$; CD8+: z = 2·26, $P<0·05$; and CD4/CD8: z = 2·27, $P<0·05$). There were significant associations between the decrease in the T lymphocyte subpopulations and the degree of fetal acidaemia.

Conclusions Growth retarded fetuses demonstrate immune abnormalities that could be attributed to intrauterine starvation.

In postnatal life, protein–energy malnutrition is associated with impaired cell mediated immune responses and increased susceptibility to infection (Chandra 1991; Schlesinger & Uauy 1991). It is generally considered that in this condition the immunosuppression results primarily from involution of the T-cell system, in particular the decrease in T-helper to T-suppressor/cytotoxic (CD4/CD8) cell ratio (Smythe *et al.* 1971; Chandra 1983).

In prenatal life, intrauterine growth retardation (IUGR) is associated with altered concentrations of fetal blood metabolites which are similar to findings in postnatal malnutrition (Economides & Nicolaides 1989; Economides *et al.* 1989, 1990). Furthermore, IUGR is associated with fetal polycythaemia, erythroblastosis, thrombocytopenia and leucopenia (Van der Hof & Nicolaides 1990; Davies *et al.* 1991; Snijders *et al.* 1993). These factors are thought to contribute to the increased susceptibility to infection of affected infants, although the exact mechanism of this defect has not been clarified (Davies *et al.* 1991). The aim of the present study was to investigate the extent to which IUGR due to placental insufficiency is associated with alterations in fetal lymphocyte subpopulations.

Subjects and methods

Fetal lymphocyte subpopulations were measured in 19 small for gestational age, chromosomally and anatomically normal fetuses referred to our unit. In all cases, ultrasound examination had demonstrated that the fetal abdominal circumference was below the 5th centile for gestation, and Doppler ultrasound investigation of the uterine and umbilical arteries was suggestive of placental insufficiency. Continuous wave Doppler studies (Dopptek Ltd, Chichester, UK) of the uterine and umbilical arteries were performed immediately before cordocentesis. An early diastolic notch in the waveform from at least one of the uterine arteries, or absent end-diastolic frequencies in the waveform from the umbilical artery, were regarded as evidence suggestive of placental insufficiency (Nicolaides *et al.* 1988; Aristidou *et al.* 1990).

Gestation at cordocentesis was 24 to 37 weeks, as determined from the maternal menstrual history or an ultrasonographic scan performed in early pregnancy. Cordocentesis was carried out 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); the full blood count was determined using a Coulter S-Plus counter (Coulter Electronics, Luton, UK). Blood films were stained by the May-Grünwald-Giemsa method for the differential cell count. Blood samples (750 μl) were also collected into heparinised syringes for measurement of pH (Radiometer ABL 330, Copenhagen, Denmark) and enumeration of lymphocyte subpopulations.

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Table 1. List of the monoclonal antibody panel used to enumerate fetal lymphocyte subpopulations, showing cluster designation (CD no.), alternative nomenclature and reactivity/specificity.

CD no.	Alternative nomenclature	Reactivity/specificity
CD3	Leu 4, UCHT1, OKT3	T cell receptor, Pan T cell marker
CD4	Leu 3a	T helper/inducer lymphocytes
CD8	Leu 2a	T suppressor/cytotoxic lymphocytes
CD19	Leu 12	Pan-B lymphocyte marker
CD16	Leu 11	NK (CD3-) cells
CD56	Leu 19, NKH-1	NK (CD3-) cells and T (CD3+) lymphocytes

Fluorescein-isothiocyanate (FITC) or Phycoerythrin (PE) conjugated monoclonal antihuman antibodies (Becton Dickinson UK Ltd., Oxford, UK) were used for simultaneous two-colour determination of lymphocyte subpopulations using CD45-FITC/CD14-PE, CD3-FITC/CD19-PE, CD4-FITC/CD8-PE and CD3-FITC/CD16-PE & CD56-PE (Table 1.). The whole blood method was used for staining the cells with monoclonal antibody (Caldwell & Taylor 1986). Cytometric analysis was carried out using a FACScan and Consort 32 software (Becton Dickinson UK Ltd, Oxford, UK). 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/leucocyte marker) to ascertain that cells were lymphoid in origin. Control staining of fetal cells with antimouse 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 abdominal circumference, blood gases, T-cell, B-cell and NK lymphocyte subpopulations change with gestation (Nicolaides *et al.* 1989; Thilaganathan *et al.* 1992, 1993a,b), the values obtained from the growth retarded fetuses were expressed as the number of standard deviations by which the individual values differed from the appropriate normal mean for gestation (Δ values, SD). The Mann-Whitney *U* test was applied to determine if the mean values in the IUGR fetuses were significantly different from the appropriate normal mean for gestation. Regression analysis was used to determine the significance of any association between Δ pH and Δ values for other variables.

Results

In the 19 IUGR fetuses, the mean abdominal circumference (AC), pO_2 and pH were significantly lower than the respective normal mean for gestation (AC: $z = 3{\cdot}80$, $P<0{\cdot}001$; pH: $z = 3{\cdot}68$, $P<0{\cdot}001$; pO_2: $z = 3{\cdot}80$, $P<0{\cdot}01$) (Fig. 1). The mean T (CD3+), B (CD19+), T-helper (CD4+), T-suppressor/cytotoxic (CD8+) cell counts and the CD4 to CD8 ratio in the IUGR fetuses were significantly lower than the respective normal mean for gestation (CD3+: $z = 3{\cdot}66$, $P<0{\cdot}001$; CD19+: $z = 2{\cdot}18$, $P<0{\cdot}05$; CD4+: $z = 3{\cdot}76$, $P<0{\cdot}001$; CD8+: $z = 2{\cdot}26$, $P<0{\cdot}05$ and CD4/CD8: $z = 2{\cdot}27$, $P<0.05$) (Figs 2 and 3). The mean natural killer (CD16+/CD56+) cell count in the IUGR fetuses was not significantly different from the

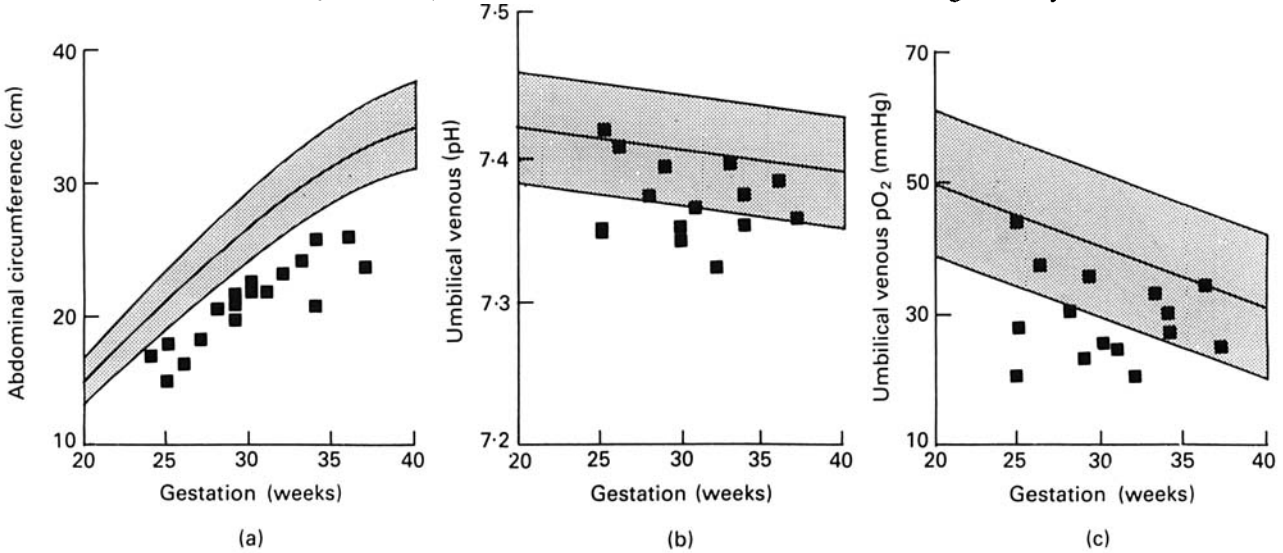

Fig. 1. The abdominal circumference, umbilical venous pH and pO_2 in the 19 growth retarded fetuses plotted on the appropriate reference range (mean, 5th and 95th centiles) for gestation.

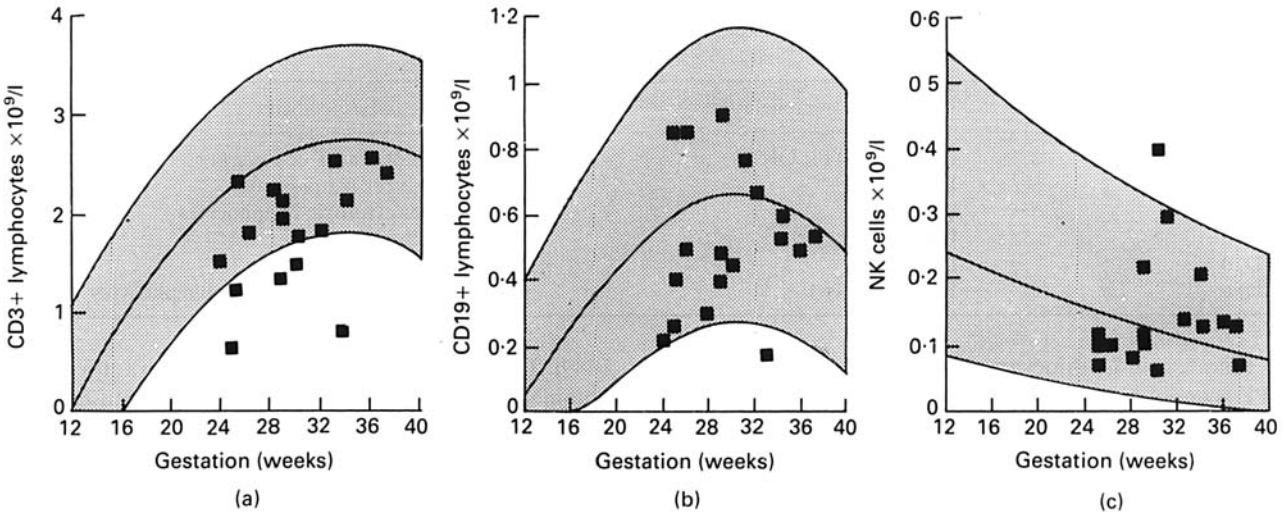

Fig. 2. The T (CD3+), B (CD19+) lymphocyte counts and NK cell number in the 19 growth retarded fetuses plotted on the appropriate reference range (mean, 5th and 95th centiles) for gestation.

normal mean for gestation (z = 0·03) (Fig. 2). There were significant associations between Δ pH and Δ CD3+ (r = 0·707, $P<0·001$), Δ CD4+ (r = 0·531, $P<0·05$) and Δ CD8+ (r = 0·618, $P<0·01$) (Fig. 4).

Discussion

This study has demonstrated that placental insufficiency is associated with fetal immune abnormalities. The findings that in growth retarded fetuses there is a greater involution in T rather than B lymphocyte number and a fall in the CD4 to CD8 ratio are consistent with those observed in malnourished children and adults (Smythe *et al.* 1971; Chandra 1983). In postnatal malnutrition, impairment in cell mediated immune responses has been shown to be due to a decrease in T-cell number rather than function (Beatty & Dowdle 1978), and T-cell counts are used to monitor the degree of immunosuppression and its recovery with refeeding (Chandra 1991). The importance of T lymphoctyes to a healthy immune response is shown in HIV infection, in which a decrease in the CD4 to CD8 ratio is associated with recurrent intractable infections and death (Stein *et al.* 1992).

The most likely explanation for the observed changes in lymphocyte subpopulations is deficiency in essential nutrients. Supportive evidence is provided by the finding of a significant association between T-cell lymphopenia and fetal acidaemia, a marker of placental insufficiency. Previous studies on blood obtained by cordocentesis from growth retarded fetuses have demonstrated abnormalities in carbohydrate, fat and protein metabolism which are probably the consequence of impaired placental perfusion and oxygenation (Economides & Nicolaides 1989; Economides *et al.* 1989, 1990). Animal studies have also

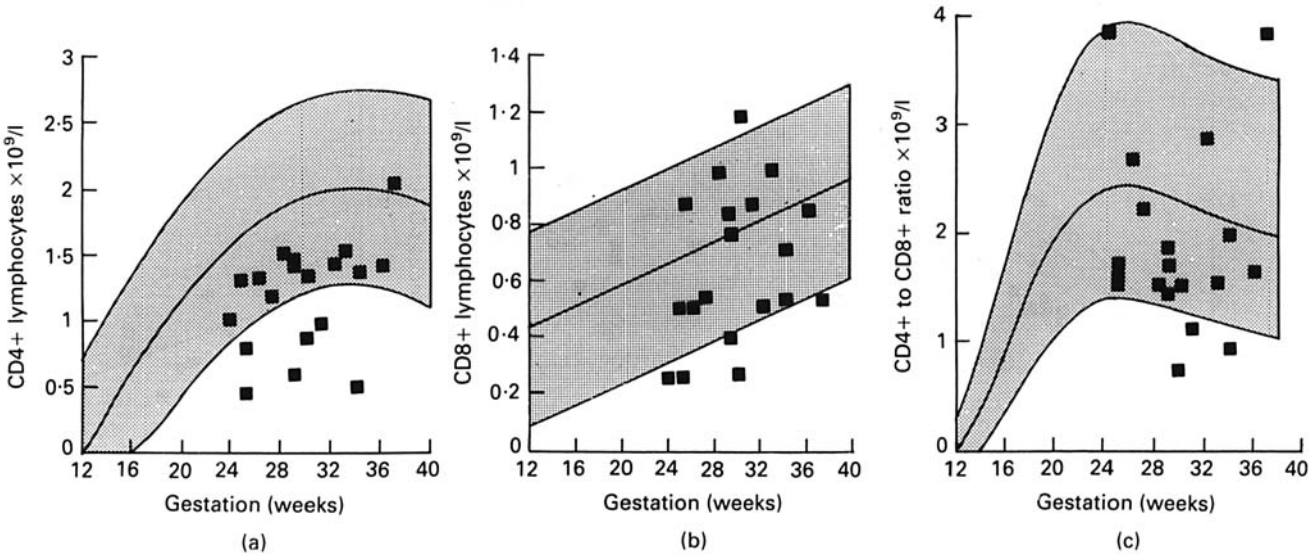

Fig. 3 The T-helper (CD4+), T-suppressor/cytotoxic (CD8+) lymphocyte counts and the CD4+ to CD8+ ratio in the 19 growth retarded fetuses plotted on the appropriate reference range (mean, 5th and 95th centiles) for gestation.

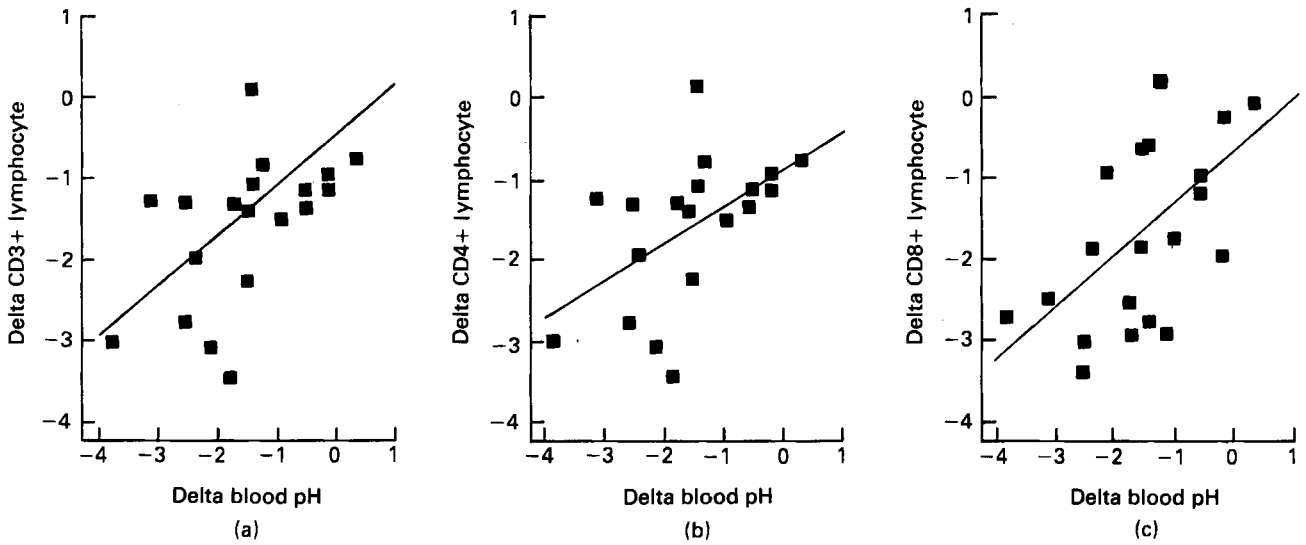

Fig. 4. Individual values and regression lines showing the relation between Δ pH and Δ CD3+, Δ CD4+ and Δ CD8+ counts in the 19 growth retarded fetuses. Delta values are the number of standard deviations by which the individual values differed from the appropriate normal mean for gestation. The most lymphopenic fetuses were also acidaemic.

shown that similar alterations in lymphocyte subpopulations may occur with isolated deficiencies of vitamins A, B6, C and E, zinc, copper, iron and various amino acids (Beisel 1982; Chandra & Dayton 1982).

Although in postnatal malnutrition refeeding results in complete recovery of immune responses, previous studies of growth retarded infants have shown that they may still be immunosuppressed at one to two years of age (Chandra *et al.* 1977). Recently, Barker *et al.* (1992) reported an association between low birthweight and the development of diabetes mellitus and ischaemic heart disease in later life. They postulated that these diseases were the consequence of the adverse effects of nutrient and oxygen deprivation at critical stages of organ development. The extent to which intrauterine starvation causes irreversible maldevelopment of the fetal immune system and increased susceptibility to infection, autoimmune disease and even malignancy in later life remains to be determined.

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