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FETAL AND NEONATAL MEDICINE

Fetal immunological and haematological changes in intrauterine infection

BASKARAN THILAGANATHAN *Research Fellow*, STEVE G. CARROLL *Research Fellow*, NICHOLAS PLACHOURAS *Research Fellow*, GEORGE MAKRYDIMAS *Research Fellow*, KYPROS H. NICOLAIDES *Professor and Director*

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

ABSTRACT

Objective To study fetal immunological and haematological changes to intrauterine infection.

Design In 37 pregnancies at risk of intrauterine infection, fetal blood obtained by cordocentesis at 20 to 36 weeks gestation was used for differential leucocyte counts, platelet count, enumeration of lymphocyte subpopulations, and neutrophil adhesion receptor expression.

Setting Harris Birthright Research Centre for Fetal Medicine, London.

Results All four fetuses with viral infections had platelet counts below the 5th centile and three had natural killer (NK) cell counts greater than the 95th centile of the normal range. Similarly, all five fetuses with bacterial or candidal infection had neutrophil counts greater than the 95th centile of the normal range; lymphocyte subpopulations were normal.

Conclusions In pregnancies complicated by intrauterine infection, fetuses exhibit NK cell lymphocytosis and thrombocytopenia in response to viraemia, and neutrophilia in response to bacteraemia from at least 21 weeks gestation.

In normal fetuses, the lymphocyte count increases linearly with gestation, and at 20 weeks the levels are approximately 50% of those at term (Davies *et al.* 1992). The relatively high numbers of lymphocytes from early pregnancy may be reconciled with the need to acquire immunological tolerance and antigen recognition functions. The latter may be essential in combating viral infections which can cross the placenta. Neutrophil counts are very low (approximately 10% of those at term) until 32 weeks and increase exponentially thereafter to reach adult levels at term (Davies *et al.* 1992). Presumably the placenta acts as an effective barrier to most bacteria (Klein & Remington 1990), and therefore the acquisition of host defence mechanisms directed against bacterial infection is only necessary in the third trimester in preparation for extrauterine life. Supportive evidence for this hypothesis is provided by the results of flow cytometric studies that reported the presence of phenotypically mature lymphocytes from at least mid-gestation and phenotypically mature neutrophils only at term (Carr *et al.* 1992; Thilaganathan *et al.* 1992, 1993a, b; Török *et al.* 1993).

Correspondence: Professor K. H. Nicolaides, The Harris Birthright Research Centre for Fetal Medicine, King's College Hospital School of Medicine, Denmark Hill, London SE5 8RX, UK.

The aim of the present study was to investigate the extent to which intrauterine viral and bacterial infections are associated with alterations in fetal leucocyte and platelet counts, lymphocyte subpopulations and neutrophil adhesion receptor expression.

Subjects and methods

Leucocyte count and lymphocyte subpopulations were determined in fetal blood obtained by cordocentesis at 20 to 36 weeks gestation from 37 pregnancies at risk of intrauterine infection. In 14 women there were positive maternal blood virus-specific IgM titres for toxoplasmosis ($n = 7$), rubella virus ($n = 4$), cytomegalovirus ($n = 2$), or parvovirus ($n = 1$). The cases of toxoplasmosis and rubella virus infection were identified at routine antenatal screening and ultrasonographic examination demonstrated normal fetal anatomy and size for gestation. The cases of cytomegalovirus and parvovirus infection were identified after specific testing because ultrasound examination had demonstrated fetal hydrocephalus and hydrops respectively. In all cases of suspected viral infection fetal serum was tested for the appropriate virus specific IgM, and four were positive (two for rubella virus, one each for cytomegalovirus and parvovirus). In the cases of toxo-

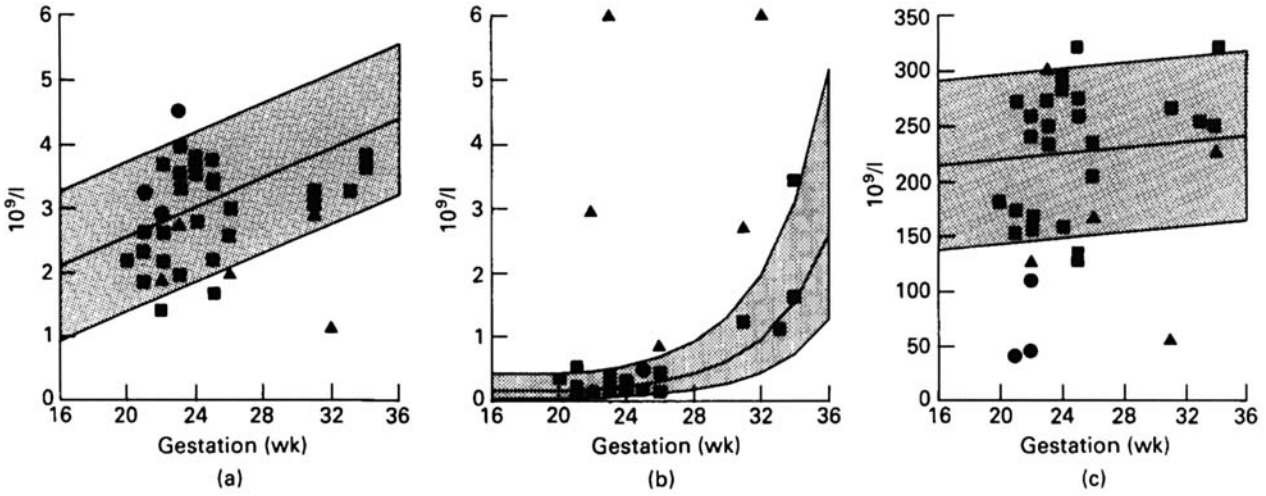

Fig. 1. The lymphocyte, neutrophil and platelet counts in the 28 noninfected (■), 4 virally infected (●) and 5 bacterially infected (▲) fetuses plotted on the appropriate reference range (mean, 5th and 95th centiles) for gestation.

plasmosis fetal blood was tested for specific IgM and both fetal blood and amniotic fluid were inoculated into mice; all cases were negative (Holliman *et al.* 1991).

Fetal blood also was examined from 23 pregnancies with preterm prelabour rupture of membranes (PPROM). The diagnosis of PPROM was confirmed by the ultrasonographic demonstration of decreased or absent amniotic fluid and the visualisation of Nitrazine positive fluid in the vagina. In all cases, fetal blood was inoculated into aerobic and anaerobic blood culture bottles (Bactec, Becton-Dickinson, Towson, USA) and in five they were positive (one each for *Enterobacter* spp, *Citrobacter* spp, *Streptococcus agalactiae*, *Streptococcus milleri* and *Candida albicans*).

Fetal full blood counts were performed (Coulter S-Plus counter, Coulter Electronics, Luton, UK), and blood films were stained by the May-Grünwald-Giemsa method for the differential cell count. Blood samples (500 µl) were also were collected into heparinised syringes for enumeration of lymphocyte subpopulations in all cases (Caldwell & Taylor 1986), and into 500 µl of 0·4% formaldehyde–phosphate-buffered saline (PBS, Dulbecco's, Oxoid) for estimation of neutrophil adhesion receptor expression in 12 PPROM cases (Hamblin *et al.* 1992).

Fluorescein–isothiocyanate (FITC) or Phycoerythrin (PE) conjugated monoclonal anti-human antibodies (Becton Dickinson UK Ltd., Oxford, UK) were used for simultaneous two-colour determination of lymphocyte subpopulations and neutrophil adhesion receptor expression using CD45+CD14, CD3+CD19, CD4+CD8, CD3+CD16&CD56, CD18 and CD11b. Cells were stained with monoclonal antibodies using protocols described previously (Caldwell & Taylor 1986; Hamblin *et al.* 1992). 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 monocytes and platelets. Gated cells were analysed with CD14+CD45 (monocyte/leucocyte marker) to ascertain that cells were lymphoid or myeloid 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 less than 1% were obtained. A minimum of 5000 cells were analysed to calculate the percentage and the mean fluorescence intensity (MFI) of each subset. The absolute number of cells was derived from the total nucleated cell count, differential count and the percentage of lymphocytes on the blood film. The relative fluorescence intensity (RFI) was calculated using the formula RFI = antilog (MFI/number of channels per decade) (Finn 1993).

Results

The results of the various investigations were considered in three groups: noninfected ($n = 28$), virally infected ($n = 4$), and bacterially infected ($n = 5$). In the noninfected group, compared with the appropriate normal mean for gestation (Figs 1 and 2), there were no significant differences in lymphocyte count ($t = -1{\cdot}53$), neutrophil count ($t = 1{\cdot}65$), platelet count ($t = -0{\cdot}07$), NK cell number ($t = 1{\cdot}83$), CD4+/CD8+ ($t = -1{\cdot}94$) or neutrophil adhesion receptor expression ($n = 11$; CD18: $t = -0{\cdot}12$; CD11b: $t = 0{\cdot}45$).

In the virally infected group, all four fetuses were thrombocytopenic. The neutrophil count was normal in all four cases, but the lymphocyte count was elevated in the fetus with cytomegalovirus infection (Fig. 1). The NK cell number was increased in all but one of the cases, and there was a tendency for a decrease in the CD4+ to CD8+ ratio (Fig. 2). In this group there were two intrauterine deaths, one termination of pregnancy, and one livebirth with confirmed congenital rubella infection resulting in profound deafness at nine months of age.

In the bacterially infected group, all fetuses were neutrophilic. The lymphocyte and platelet counts were decreased in two of the five cases (Fig. 1). The NK cell number and CD4+ to CD8+ were within the normal range, and the neutrophil adhesion receptor (CD18 and CD11b) expression was normal in the one case, a 23 week

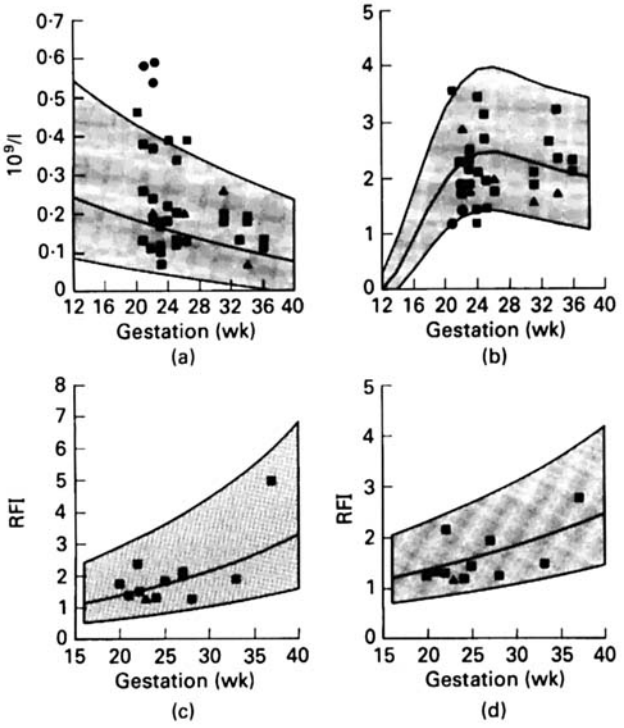

Fig. 2. The NK cell number, CD4+ to CD8+ ratio, neutrophil adhesion receptor (CD18 and CD11b) relative fluorescence intensity (RFI) in the 28 noninfected (■), 4 virally infected (●) and 5 bacterially infected (▲) fetuses plotted on the appropriate reference range (mean, 5th and 95th centiles) for gestation (Van der Hof & Nicolaides 1990; Favies *et al.* 1992).

fetus with *Citrobacter* spp infection (Fig. 2), that was tested. In this group there were three terminations of pregnancy and two preterm livebirths.

Discussion

The data of this study demonstrate that intrauterine bacterial infection is associated with fetal neutrophilia from at least 22 weeks gestation. This is in contrast to the finding of neutropenia in septic infants (Christensen *et al.* 1982; Mease 1990). The explanation for this apparent discrepancy is unlikely to be the effect of labour and delivery; labour is associated with an increase rather than decrease in fetal leucocyte count (Thilaganathan *et al.* 1994). We have previously shown that when PPROM is associated with fetal infection, delivery occurs within five days (personal communication), and by implication our finding of neutrophilia represents the acute response to infection (Hogg 1992). Presumably, mobilisation of neutrophils results in exhaustion of the neutrophil storage pool and with prolonged infection neutropenia ensues (Christensen & Rothstein 1981; Mease 1990). In this respect, the fetus may not be dissimilar to the adult where infection is associated with neutrophilia, but when the infection is overwhelming there is neutropenia.

The effectiveness of the response to infection is determined not only by the degree of neutrophilia, but also by the functional capacity of the neutrophils. The latter, which includes polymorphonuclear adhesion, chemotaxis and transmigration through the endothelium, is closely associated with upregulation of mainly CD18 and CD11b neutrophil adhesion receptor expression (Arnaout 1990). Previous studies have demonstrated that neonatal neutrophil function and adhesion receptor expression are impaired (Carr *et al.* 1992; Török *et al.* 1993). This is consistent with our finding that in an infected fetus at 23 weeks gestation there was no upregulation of neutrophil CD18 and CD11b expression.

Congenital viral infection is associated with increase in NK cell number and decrease in CD4+ to CD8+ ratio, demonstrating that fetuses are capable of altering lymphocyte subpopulations in an adult-type manner (Luft *et al.* 1984) from at least 21 weeks gestation. This finding is consistent with that of Hohlfeld *et al.* (1990) who reported an overall decrease in the CD4+ to CD8+ ratio in nine cases of congenital toxoplasmosis infection; they did not measure NK cell numbers. In adults with rubella infection, alteration in CD4+ to CD8+ ratio only occurs during the acute phase of the infection and returns to normal shortly thereafter (Hyypia *et al.* 1984). In contrast, in individuals with congenital rubella syndrome the changes persist for several decades and may be permanent (Rabinowe *et al.* 1986). It is likely that rubella infection during immunological development causes irreversible damage to the immune system and this may predispose to autoimmune disease in postnatal life (Rabinowe *et al.* 1986).

Thrombocytopenia was observed in all four virally infected fetuses and is consistent with the finding that thrombocytopenic petechiae is the commonest symptom in infants with congenital viral infections (Pass *et al.* 1980). Since, the presence of symptoms in congenitally infected infants is the main determinant of poor outcome, intrauterine thrombocytopenia may help identify those infected fetuses that are also at such high risk. This finding is compatible with those of a previous study (Holfeld *et al.* 1991) which reported that of five cases of intrauterine cytomegalovirus infection, one with fetal thrombocytopenia died in the neonatal period; of the remaining four with normal platelet counts, three were asymptomatic and one had hearing loss when tested at birth.

This study has demonstrated immunological and haematological alterations in fetuses with intrauterine infection. The extent to which these changes will help distinguish fetuses with a poor prognosis from those with a normal outcome, remain to be determined.

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