British Journal of Obstetrics and Gynaecology
January 1993, Vol. 100, pp. 60–62

FETAL AND NEONATAL MEDICINE

Abnormal fetal immunological development in Down's syndrome

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ABSTRACT

Objective To investigate the intrauterine development of the immune system in Down's syndrome.

Design Cross sectional study.

Setting Harris Birthright Research Centre for Fetal Medicine, London, UK.

Subjects 16 fetuses with Down's syndrome and 104 fetuses with a normal karyotype at 17–24 weeks gestation.

Main outcome measures Flow cytometry was used to enumerate T (CD3+), B (CD19+) and NK (CD3− & CD16+/CD56+) lymphocyte subpopulations in fetal blood obtained by cordocentesis.

Results The median numbers of T, B and NK lymphocytes in fetuses with Down's syndrome ($1{\cdot}52 \times 10^9$/l, $0{\cdot}08 \times 10^9$/l, and $0{\cdot}10 \times 10^9$/l, respectively) were significantly lower than in the chromosomally normal fetuses (T lymphocytes: $1{\cdot}98 \times 10^9$/l, $z = 3{\cdot}04$, $P<0{\cdot}01$; B lymphocytes: $0{\cdot}50 \times 10^9$/l, $z = 5{\cdot}84$, $P<0{\cdot}0001$; and NK lymphocytes: $0{\cdot}19 \times 10^9$/l, $z = 3{\cdot}14$, $P<0{\cdot}01$).

Conclusion These data demonstrate that in Down's syndrome, there is abnormal intra-uterine development of the immune system.

Babies with Down's syndrome are at increased risk of infection, autoimmune disease and malignancy presumably because of immunological deficiencies, such as decreased numbers of circulating T and B lymphocytes, impaired phagocytosis and reduced cell-mediated and humoral immune responses (Franceschi *et al.* 1981; Lockitch *et al.* 1987; Cossarizza *et al.* 1991). It is generally assumed that these immunological derangements may be the consequence of various postnatal factors including precocious aging, persistence of hepatitis B surface antigen, zinc deficiency and premature failure of the thymus (Franceschi *et al.* 1981; Fabris *et al.* 1984; Lockitch *et al.* 1987). The aim of this study is to investigate whether the immunological deficiencies are indeed the consequence of postnatal factors, or whether they are present during intrauterine life.

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Subjects and methods

Fetal blood samples were obtained by cordocentesis from 130 pregnancies undergoing prenatal diagnosis at 17–24 weeks gestation. The fetal karyotype subsequently was found to be normal in 104 fetuses and 16 were affected by Down's syndrome. The indications for fetal karyotyping were advanced maternal age ($n = 14$), low maternal serum alpha–fetoprotein ($n = 32$), and minor fetal malformations such as choroid plexus cysts or hydronephrosis ($n = 84$). In all samples, the fetal abdominal circumference and haemoglobin concentration were within the appropriate reference range for gestation.

The study was cross sectional with gestation being determined from the menstrual history, confirmed by an ultrasound scan in early pregnancy. Cordocentesis was performed without maternal sedation or fetal paralysis, and umbilical venous blood was obtained from all fetuses. Kleihaur-Betke testing confirmed that all blood samples were of fetal origin.

Fetal blood samples (180 µl) were collected into 20 µl of isotonic edetic acid solution (0·5 mmol/l in 0·15 mmol/l

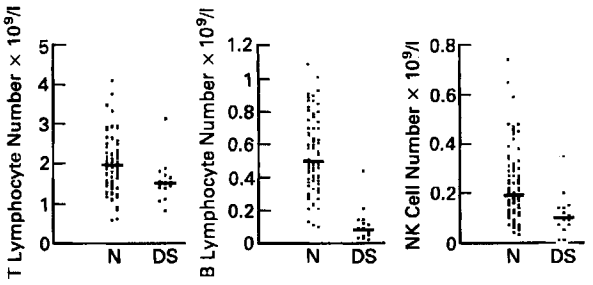

Fig. 1. Total T lymphocyte, B lymphocyte and NK cell numbers ($\times 10^9$/l) in the 104 chromosomally normal (N) and 16 Down's syndrome (DS) fetuses. The horizontal bar represents the median value for each group.

NaCl) and the full blood count was determined using a Coulter S-Plus counter (Coulter Electronics, Luton, UK). Blood films were stained by the May-Grunwald-Giemsa method for the differential nucleated cell count. Blood samples (250 µl) were collected into heparinised syringes for measurement of oxygen tension and pH (Radiometer ABL 330, Copenhagen, Denmark). Blood samples (0·5 ml) were also collected into heparinised syringes for enumeration of fetal lymphocyte subsets which was performed on the day of sampling.

Flow cytometry

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 using CD45–FITC/CD14–PE (monocyte/pan–leucocyte marker), CD3–FITC/CD19–PE (T and B lymphocytes) and CD3–FITC/CD16–PE & CD56–PE (NK cells and cytotoxic T lymphocytes). 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, Oxford, UK) as previously described (Thilaganathan *et al.* 1992).

Statistical analysis

Mann-Whitney tests were used to determine the significance of any differences between the two groups.

Results

In the fetuses with Down's syndrome, the median numbers of T (CD3+), B (CD19+) and NK (CD3– & CD16+/CD56+) lymphocytes were significantly lower than the respective values in the chromosomally normal fetuses (Fig. 1, Table 1). The gestational age of the two groups was not significantly different (Table 1).

Discussion

The findings of this study, (that fetuses with Down's syndrome have a decreased number of circulating T and B lymphocytes), are compatible with data from postnatal studies in children and adults with Down's syndrome and demonstrate that these immunological deficiencies occur prenatally (Lockitch *et al.* 1987; Cossarizza *et al.* 1991). By comparison, the number of circulating NK cells in fetuses with Down's syndrome is decreased, whereas the number in affected adults is greatly increased (Cossarizza *et al.* 1991), suggesting that this increase may indeed by due to postnatal events (Abo *et al.* 1982).

The observed immunological defects in Down's syndrome are likely to be the consequence of the extra chromosome 21 resulting in the overproduction of certain proteins. For example, copper zinc superoxide dismutase (CuZnSOD), which is encoded on chromosome 21, is overactive in patients with Down's syndrome, and could cause abnormal immunological development (Porstmann *et al.* 1991). Overexpression of CuZnSOD has been shown to result in decreased zinc concentration, reduced bioavailability of thymulin, and suppressed cellular production of prostaglandin E_2 and D_2 (Lockitch *et al.* 1987; Cossarizza *et al.* 1991; Minc-Golomb *et al.* 1991). All of these factors have been implicated in the aetiology of abnormal immunological function (Chandra & Dayton 1979; Fabris *et al.* 1984; Johnston 1988). Alternatively, overexpression of CD18, a leucocyte intergrin which is also encoded by a gene on chromosome 21, has been shown to result in defi-

Table 1. Lymphocyte subpopulations in normal and Down's syndrome fetuses. The median and interquartile range of the gestational age, number of cells ($\times 10^9$/l) in each subpopulation and the significance of any differences (NS = not significant) between the two groups are shown.

	Normal fetuses (n = 104) median (range)	Down's Syndrome (n = 16) median (range)	Significance of differences
Gestational age (weeks)	21 (20–22)	21 (19–23)	NS
No. of T lymphocytes	1·98 × 10^9/l (1·57–2·29 × 10^9/l)	1·52 × 10^9/l (1·41–1·71 × 10^9/l)	z = 3·04 P<0·01
No. of B lymphocytes	0·50 × 10^9/l (0·38–0·67 × 10^9/l)	0·08 × 10^9/l (0·04–0·14 × 10^9/l)	z = 5·84 P<0·0001
No. of NK cells	0·19 × 10^9/l (0·12–0·29 × 10^9/l)	0·10 × 10^9/l (0·07–0·13 × 10^9/l)	z = 3·14 P<0·01

cient cell-to-cell adhesion and interaction (Taylor *et al.* 1988). This functional deficit results in decreased cytotoxicity and lymphoid hypoplasia (Hildreth *et al.* 1983; Nunoi *et al.* 1988).

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Received 13 May 1992
Accepted 17 July 1992