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ABNORMAL IMMUNOLOGICAL DEVELOPMENT IN FETUSES WITH TRISOMY 18

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SUMMARY

Flow cytometry was used to enumerate the lymphocyte subpopulations in fetal blood obtained by cordocentesis from eight trisomy 18 fetuses at 20–36 weeks' gestation. Compared with values in chromosomally normal fetuses, in trisomy 18 the mean T- and natural killer (NK) cell counts were significantly lower ($t = -7{\cdot}63$, $P<0{\cdot}001$ and $t = -3{\cdot}58$, $P<0{\cdot}01$, respectively); the mean B-cell count was not significantly different ($t = -1{\cdot}32$). These findings demonstrate that in trisomy 18 there is abnormal intrauterine development of the immune system.

KEY WORDS—Fetal blood, cordocentesis, lymphocyte subsets, trisomy 18, fetal immunology.

INTRODUCTION

Flow cytometric studies of fetal blood obtained by cordocentesis have demonstrated that during normal human immune development, the fetal T- and B-lymphocyte counts increase exponentially with gestation to reach a plateau in the third trimester, whereas the number of natural killer (NK) cells decreases with gestation (Thilaganathan *et al.*, 1992, 1993a,b). Study of fetuses with trisomy 21 has shown that the numbers of all three lymphocyte subsets are decreased, and these immunological defects were thought to be the consequence of the extra chromosome 21 resulting in overproduction of certain proteins (Thilaganathan *et al.*, 1993c). The aim of this study was to investigate the immunological development of fetuses with trisomy 18 by examining peripheral blood lymphocyte subpopulations.

PATIENTS AND METHODS

Fetal blood samples were obtained by cordocentesis from eight fetuses where ultrasonography revealed multiple malformations, including strawberry-shaped skull, choroid plexus cysts, micrognathia, cleft lip and palate, diaphragmatic hernia, exomphalos, overlapping fingers, and talipes. Gestational age was determined from the maternal menstrual history and confirmed by an ultrasound scan in early pregnancy. Kleihauer–Betke testing demonstrated that all blood samples were fetal and cytogenetic analysis confirmed the diagnosis of trisomy 18 in all these cases.

Fetal blood samples (180 μl) were collected into 20 μl of isotonic edetic acid solution (0·5 mmol/l in 0·15 mmol/l NaCl) and the full blood count was determined using a Coulter S Plus counter (Coulter Electronics, Luton, U.K.). Blood films were stained by the May–Grunwald–Giemsa method for the differential nucleated cell count. Blood samples were also collected into heparinized syringes for enumeration of fetal lymphocyte subsets, which was performed on the day of sampling. Flow cytometric analysis was performed using a panel of conjugated antibodies (Table I), as previously described (FACScan and Consort 32 software; Becton Dickinson, Oxford, U.K.) (Thilaganathan *et al.*, 1993c; Caldwell and Taylor, 1986).

Statistical analysis

In normal pregnancy, fetal lymphocyte subpopulations change with gestation (Thilaganathan

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Table I—List of the monoclonal antibody panel used to enumerate fetal lymphocyte subpopulations, showing cluster designations (CD No.), alternative nomenclature, and reactivity/specificity

CD No./name	Alternative nomenclature	Reactivity/specificity
CD3	Leu 4, UCHT1, OKT3	T-cell receptor, pan T-cell marker
CD19	Leu 12	Pan B-cell marker
CD16	Leu 11	NK (CD3 −) cells
CD56	Leu 19, NKH-1	NK (CD3 −) cells and cytotoxic T (CD3+) lymphocytes

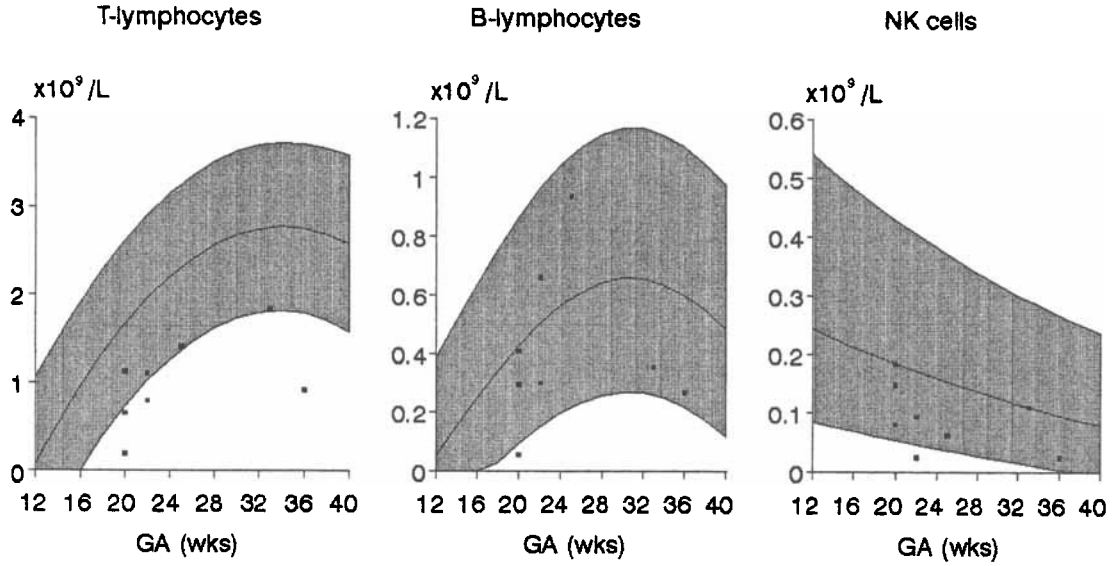

Fig. 1—Fetal T-, B-, and NK lymphocyte numbers in the eight trisomy 18 fetuses plotted on the appropriate reference range (mean, fifth, and 95th centiles) for gestation

et al., 1992, 1993a,b). The values obtained from the fetuses with trisomy 18 were expressed as the number of standard deviations by which the individual values differed from the appropriate normal mean for gestation (delta values, SDs). A two-tailed Student's *t*-test was applied to determine whether the mean values in the trisomy 18 fetuses were significantly different from the appropriate normal mean for gestation.

RESULTS

The mean T- and NK-cell counts in the trisomy 18 fetuses were significantly lower than the appropriate normal mean for gestation (Fig. 1: T cells: $t = -7{\cdot}63$, $P<0{\cdot}001$; NK cells: $t = -3{\cdot}58$, $P<0{\cdot}01$). The mean B-cell count was not significantly different from the appropriate normal mean for gestation (Fig. 1: $t = -1{\cdot}32$).

DISCUSSION

Trisomy 18, with an incidence of about 0·3 per 1000 newborns, is the second most common multisystem malformation syndrome. However, most of the affected children die soon after birth and only 10 per cent survive longer than a year (Smith, 1988). In the latter group, infection is a major cause of mortality and morbidity (Hecht, 1981; Van Dyke and Allen, 1990). The findings of this

study demonstrate that in trisomy 18 there is impaired intrauterine immunological development and they may provide a possible explanation for the postnatal susceptibility to infections in these children. Similarly, individuals with Down's syndrome are also at high risk of infection, autoimmune disease, and malignancy, which have been attributed to abnormal immunological development (Cossarizza *et al.*, 1991; Franceschi *et al.*, 1981; Thilaganathan *et al.*, 1993c).

In fetal trisomy 18, the major deficit in lymphocyte subpopulations is a decrease in T-cell counts. This differs from trisomy 21, where the predominant abnormality is a decrease in B cells. The underlying mechanism for the immunological abnormalities of fetuses with Edward's syndrome is unknown but, as in Down's syndrome, it may be the consequence of overproduction of certain enzymes encoded by genes in the extra chromosome.

REFERENCES

Caldwell, C.W., Taylor, H.M. (1986). A rapid no wash technique for immunophenotypic analysis by flow cytometry, *Am. J. Clin. Pathol.*, **86,** 600–607.

Cossarizza, A., Ortolani, C., Forti, E., Montagnani, G., Paganelli, R., Zannotti, M., Marini, M., Monti, D., Franceschi, C. (1991). Age-related expansion of functionally inefficient cells with markers of natural killer activity in Down's syndrome, *Blood*, **77,** 1263–1270.

Franceschi, C., Licastro, F., Chiricolo, M., Bonetti, F., Zannotti, M., Fabris, N., Mocchegiani, E., Fantini, M., Paolucci, P., Masi, M. (1981). Deficiency of autologous mixed lymphocyte reactions and serum thymic factor level in Down's syndrome, *J. Immunol.*, **126,** 2161–2164.

Hecht, F. (1981). Who will survive with trisomy 13 or 18?: a call for cases ten years old or above, *Am. J. Med. Genet.*, **10,** 417–418.

Smith, D.W. (1988). *Recognizable Patterns of Human Malformation*, 4th edn, Philadelphia: W. B. Saunders, 16–19.

Thilaganathan, B., Mansur, C.A., Morgan, G., Nicolaides, K.H. (1992). Fetal T lymphocyte subpopulations in normal pregnancy, *Fetal Diagn. Ther.*, **7,** 53–61.

Thilaganathan, B., Nicolaides, K.H., Mansur, C.A., Levinsky, R.J., Morgan, G. (1993a). Fetal B lymphocyte subpopulations in normal pregnancy, *Fetal Diagn. Ther.*, **8,** 15–21.

Thilaganathan, B., Abbas, A., Nicolaides, K.H. (1993b). Fetal blood natural killer cells in human pregnancies, *Fetal Diagn. Ther.*, **8,** 149–153.

Thilaganathan, B., Tsakonas, D., Nicolaides, K. (1993c). Abnormal fetal immunological development in Down's syndrome, *Br. J. Obstet. Gynaecol*, **100,** 60–62.

Van Dyke, D.C., Allen, M. (1990). Clinical management in long-term survivors with trisomy 18, *Pediatrics*, **85,** 753–759.