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NEUTROPHIL AND MONOCYTE β2-INTEGRIN EXPRESSION IN TRISOMIC FETUSES

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SUMMARY

Flow cytometry was used to measure neutrophil and monocyte β2-integrin expression in fetuses with trisomy 18 ($n=7$) and trisomy 21 ($n=7$) at 20–25 weeks' gestation. The values were compared with those of 112 chromosomally normal fetuses. There were no significant differences in β2-integrin expression between normal and aneuploid fetuses. These findings demonstrate that in trisomies 21 and 18, alteration in β2-integrin expression is unlikely to contribute to the pathogenesis of immunological deficiencies that have been observed in these aneuploidies both prenatally and postnatally.

KEY WORDS: cordocentesis; fetal blood; β2-integrins; aneuploidy; fetal immunology

INTRODUCTION

In postnatal life, trisomies 21 and 18 are associated with increased susceptibility to infections, which at least in part can be explained by immunological abnormalities (Van Dyke and Allen, 1990; Franceschi *et al.*, 1981). We have previously shown that in these trisomies, the postnatal alterations in lymphocyte subpopulations may be the consequence of abnormal intrauterine immunological development (Makrydimas *et al.*, 1994; Thilaganathan *et al.*, 1993). The β2-integrins (CD11/CD18) are essential for leucocyte adhesion-dependent functions, such as chemotaxis, phagocytosis, cell-mediated killing, and adherence to the endothelium (Arnaut, 1990). The aim of this study was to investigate the development of neutrophils and monocytes in fetuses with aneuploidies by examining surface β2-integrin expression, namely the antigens LFA-1 (CD11a/CD18), Mol-1 or Mac-1 (CD11b/CD18), and LeuM5 (CD11c/CD18).

PATIENTS AND METHODS

Neutrophil and monocyte β2-integrin expression was determined in 14 fetuses with trisomy 21 ($n=7$) or trisomy 18 ($n=7$) at 20–25 weeks' gestation. In these cases, fetal blood was obtained by cordocentesis for fetal karyotyping because ultrasound examination demonstrated one or more of the following defects: strawberry-shaped head, brachycephaly, holoprosencephaly, choroid plexus cysts, cystic hygromata, cardiac defects, diaphragmatic hernia, hydronephrosis, exomphalos, digital abnormalities, and talipes.

β2-integrin expression was also determined in 112 controls. In these cases, cordocentesis was performed at 20–25 weeks' gestation for prenatal diagnosis of fetal infection or hereditary blood disorders or for karyotyping because of advanced maternal age, abnormal maternal serum biochemistry, or ultrasound findings of mild hydronephrosis or choroid plexus cysts. In all cases, the fetal karyotype was normal and the fetuses did not have the infection or blood disorder for which they were tested.

Flow cytometry

Fluorescein isothiocyanate (FITC)-conjugated purified mouse monoclonal antibodies (CD18,

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Table I—Relative fluorescence intensity (median and range) of neutrophil and monocyte β2-integrin expression in normal, trisomy 18, and trisomy 21 fetuses. The z values for the comparison between the three groups are also shown

	Normal	Trisomy 18	Trisomy 21	Normal vs. trisomy 18	Normal vs. trisomy 21
Neutrophil CD18	1·29 (1·1–2·62)	1·32 (1·26–1·49)	1·26 (1·19–1·48)	z=0·08	z=0·82
Neutrophil CD11a	1·22 (1·1–1·47)	1·22 (1·2–1·3)	1·18 (1·15–43)	z=0·78	z=0·12
Neutrophil CD11b	1·33 (1·1–2·52)	1·33 (1·15–73)	1·33 (1·24–1·49)	z=0·06	z=0·40
Neutrophil CD11c	1·22 (1·1–1·4)	1·23 (1·12–1·32)	1·20 (1·17–1·32)	z=1·40	z=0·85
Monocyte CD18	1·21 (1·1–1·49)	1·20 (1·16–1·59)	1·23 (1·12–1·3)	z=0·70	z=0·22
Monocyte CD11a	1·16 (1·09–1·37)	1·19 (1·12–1·38)	1·15 (1·12–1·34)	z=0·79	z=0·33
Monocyte CD11b	1·20 (1·09–1·37)	1·15 (1·12–1·74)	1·29 (1·12–1·31)	z=0·62	z=1·19
Monocyte CD11c	1·14 (1·09–1·31)	1·14 (1·12–1·22)	1·16 (1·12–1·24)	z=1·11	z=0·02

CD11a, CD11b, and CD11c; DAKO Ltd., High Wycombe, U.K.) were used for determination of neutrophil and monocyte adhesion receptor expression. Control staining of fetal cells with anti-mouse monoclonal IgG_{2a}-FITC and IgG_1-FITC to keyhole limpet haemocyanin (Becton Dickinson U.K. Ltd., Oxford, U.K.) was performed on each sample. Fetal blood (500 μl) was collected without anticoagulant into an equal volume of 0·4 per cent formaldehyde in HBSS and left at 37°C for 4 min. Erythrocytes were lysed by the addition of 20 ml of 0·001 M Hepes-buffered ammonium chloride (0·155 M). After incubation at 37°C for 10 min, the cells were washed twice in HBSS by centrifugation at 300 g and resuspended to 1 ml of HBSS. Aliquots (50 μl) of cells were then incubated with 10 μl of the appropriate antibody at room temperature for 20 min and then transferred to 1 per cent formaldehyde before analysis by flow cytometry (Hamblin *et al.*, 1992). This particular protocol and single staining with monoclonal antibodies were used in order to avoid sampling related upregulation of neutrophil and monocyte cell adhesion receptor expression (Hamblin *et al.*, 1992; Macey *et al.*, 1992). Cytometric analysis was performed using a FACScan and Consort 32 software (Becton Dickinson U.K. Ltd., Oxford, U.K.). The flow cytometer was calibrated for size and fluorescence every day before data acquisition using a fluorescence intensity standardization kit (Coulter Ltd., Luton, U.K.). Samples were gated using forward angle and 90° light-scattering properties to identify neutrophils and monocytes. Gated cells were analysed with CD14/CD15 (DAKO Ltd.) to ascertain that they were of monocyte/neutrophil origin. A minimum of 5000 cells were analysed and the mean fluorescence intensity (MFI) in arbitrary units was measured. The density of surface glycoproteins was measured by calculating the relative fluorescence (RFI) by using the formula RFI=antilog(MFI/number of channels per decade) (Finn, 1993).

RESULTS

The median and range of neutrophil and monocyte β2-integrin surface expression in trisomic and chromosomally normal fetuses are shown in Table I. Comparison of values by the Mann–Whitney U-test showed no significant differences between the groups.

DISCUSSION

Postnatal studies in trisomy 21 have demonstrated increased expression of LFA-1 (CD18 and

CD11a) on lymphoblastoid cells, resulting in increased adhesive function (Taylor *et al.*, 1988a,b). It was assumed that this was a gene dosage effect because the CD18 gene is located on chromosome 21 (Marlin *et al.*, 1986). However, our findings in trisomic fetuses and those of Barrena *et al.* (1992) in affected children, that LFA-1 expression in neutrophils and monocytes is normal, suggest that there may be an alternative explanation for increased LFA-1 expression on lymphoblastoid cells. Maccario *et al.* (1984) reported that in trisomy 21 there is an increase in the proportion of CD8 cells that express the CD57 antigen; these cells have been shown to have high surface expression of LFA-1 (Nishimura and Itho, 1988).

We have previously demonstrated that in normal fetuses, neutrophil and monocyte β2-integrin expression increases with gestation to reach adult levels by term (Thilaganathan *et al.*, 1995). The findings of this study indicate that in trisomies 21 and 18, fetal β2-integrin expression is normal and it is therefore unlikely that abnormalities in this system contribute to the pathogenesis of immunological deficiencies that have been observed in these aneuploidies both prenatally and postnatally.

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