Enhanced expression of the immunoregulator, p43-placental isoferritin, in Down’s syndrome placenta and fetal kidneys

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Human placental isoferritin is composed of a 43 kDa subunit and ferritin light chains. It acts as an immunosuppressive cytokine in normal gestation and in some malignant conditions. We investigated p43-placental isoferritin expression at the maternal fetal tissue interface and in fetal kidneys in Down’s syndrome (DS) compared with normal control samples. Following termination of mid-gestation pregnancies placental and fetal kidney tissue samples were collected. Immunohistochemical analysis of the specimens was performed using a monoclonal antibody generated against human p43-placental isoferritin protein (CM-H-9 mAb). Expression of p43-placental isoferritin was detected in Hofbauer cells and in the syncytial layer of placental tissue. Significantly higher numbers of positive Hofbauer cells were detected in DS placentae at 17 weeks gestation compared with the controls. The number of immunopositive Hofbauer cells decreased in DS placentae at 20 weeks gestation, 3 weeks later than in controls. In kidneys of fetuses at 17 weeks gestation, p43-placental isoferritin immunoreactivity was confined to the proximal tubules of the nephrons. DS kidneys had higher staining intensities compared with similar gestational age controls. Enhanced expression of p43-placental isoferritin was observed in DS placentae and fetal kidneys. This may explain the increased p43-placental isoferritin levels in the maternal serum of DS gestations.

Key words: Down’s syndrome/fetal kidneys/p43-isoferritin/placenta/PLIF

Introduction

Human placental ferritin is an immunosuppressive protein which acts as a downregulator of the immune response (Maymon and Moroz, 1996; Rosen, 1996). The term ‘carcinofetal isoferritin’ was proposed by Drysdale and Singer to distinguish similar biophysical characteristics of isoferritin isolated from both the conceptus and some malignant tumours (Drysdale and Singer, 1974). Placental isoferritin is a heteropolymer that consists of a 43 kDa subunit (p43) and ferritin light chains. It was identified by CM-H-9, the monoclonal antibody (mAb) which reacts exclusively with p43. Since there is no correlation between serum ferritin and p43-placental isoferritin (p43-PLF) levels, its production appears to be unrelated to maternal iron stores (Maymon and Moroz, 1996). Elevated p43-placental isoferritin levels have been measured in the serum of pregnant women throughout normal gestation, but are undetectable in the majority of healthy blood donors (Moroz et al., 1987).

The gene coding for p43 has been recently cloned and named placenta immunoregulatory ferritin (PLIF) (Moroz et al., 2002). This gene encodes an incomplete ferritin heavy chain sequence lacking the 65 C-terminal amino acids which are substituted with a novel 48 amino acid sequence (C48). The immunoregulatory and specific immunoreactivity detected by CM-H-9 mAb are attributed to C48 from which the cytokine-like domain is composed (Moroz et al., 2002).

p43-PLF has been immunohistochemically demonstrated to be strongly expressed in the villous syncytiotrophoblast and in Hofbauer cells (Maymon et al., 2000a). This expression in the syncytial layer is highest during the first trimester of pregnancy, whereas it is detected only in Hofbauer cells later in gestation. These findings suggest that p43-PLF has an immunoregulatory role during the early stages of placentation (Maymon et al., 2000a). Intense p43-PLF immunostaining has also been demonstrated in the proximal tubules of the primitive nephron and macrophages in embryos and fetuses (Maymon et al., 2000b).

Down’s syndrome (DS) children are known to have both humoral and cellular immune defects (Murphy and Epstein, 1990; Ugazio et al., 1990), an increased susceptibility to leukaemia and lymphoma (Baird et al., 1988) and an increased incidence of celiac disease (Gale et al., 1997). High serum p43-PLF levels have been detected in these pathological conditions (Moroz et al., 1987, Dinary et al., 1991; Garty et al., 1997). Accordingly, elevated maternal serum p43-PLF levels have been demonstrated both in DS gestations (Moroz et al., 2000) and in DS children (Garty et al., 1997).

The present study aims to gain insight into p43-PLF expression at the maternal–fetal tissue interface and fetal kidneys of DS pregnancies.

Materials and methods

Samples

Fetal and villous tissues were collected from 10 healthy pregnant women undergoing cervical dilation and surgical evacuation of a DS pregnancy at 17 and 20 weeks gestation. Cytogenetic analysis had verified the karyotype results in all these cases, and informed written consent had been obtained.
Figure 1. p43-placental isoferritin immunolocalization in placental villi of normal pregnancies (A–C) and in DS pregnancies (D and E). (A) Villous tissue at 8 weeks gestation in a normal pregnancy showing p43-placental isoferritin immunostaining of the syncytial layer (arrows) and in Hofbauer cells (arrow). (B) Villous tissue at 15 weeks gestation in a normal pregnancy showing p43-placental isoferritin immunostaining in Hofbauer cells (arrow). Weak staining is noted in the syncytial layer (arrows). (C) Villous tissue at 17 weeks gestation in a normal pregnancy. p43-placental isoferritin immunostaining is localized in single apparent Hofbauer cells (arrow). No immunoreactivity is observed in the syncytial layer. (D) DS placental villous at 17 weeks gestation. p43-placental isoferritin immunostaining is localized in the abundant Hofbauer cells. No immunoreactivity is observed in the syncytial layer. (E) DS placental villous at 20 weeks gestation. p43-placental isoferritin immunostaining is observed in the Hofbauer cells. No immunoreactivity is observed in the syncytial layer. (A–E) Original magnification ×400.

Figure 2. p43-placental isoferritin immunolocalization in normal kidney (A) and in DS kidney (B) obtained from fetuses at 17 weeks gestation. G = glomeruli; P = proximal tubule. (A) Weak immunostaining observed in the nephron’s proximal tubules of normal kidney. (B) Strong immunostaining located in the nephron’s proximal tubules of DS kidney. (A and B) Original magnification ×400.
from each woman before the procedures were carried out. This study was approved by the Ethics Committee of Human Research of the University College of London Hospital where it was conducted. Gestational age was confirmed by biometry scan, and fetal heart rate was detected prior to uterine evacuation. Following surgery, the retrieved villous tissues and fetal kidneys were separated by a dissecting microscope and immediately fixed in 10% buffered formalin solution. They were then embedded in paraffin, as previously described (Maymon et al., 2000a,b). The DS specimens were compared with normal control samples which had been similarly obtained over the same period of time (Maymon et al., 2000a,b).

**Immunohistochemistry**

The studied specimens were cut into consecutive longitudinal sections (3 µm in thickness) and every fifth section was immunohistochemically stained for p43-PLF. The sections were incubated with CM-H9 monoclonal antibody (mAb) generated specifically against human p43-PLF protein as previously described (Moroz et al., 1985). The sections were mounted on Super Frost/Plus glass (Menzel Glaser, Braunschweig, Germany) and processed by the labelled (strept) avidin-biotin (LAB-SA) method using Histostain™Plus Kit (Zymed, San Francisco, USA), according to the manufacturer’s instructions. The sections were treated with 3% H2O2 for 5 min, followed by incubation with normal human serum for 10 min and subsequently incubated for 1 h with a 1:150 dilution of CM-H9 mAb (1 mg/ml). In addition, consecutive sections were routinely stained using haematoxylin and eosin. Control incubations were performed by substituting non-immune serum for the primary antibody. The biotinylated second antibody was applied for 10 min, followed by incubation with horseradish peroxidase conjugated streptavidin (HRP-SA) antibody. The biotinylated second antibody was applied for 10 min, followed by incubation with horseradish peroxidase conjugated streptavidin (HRP-SA) for 10 min. After each incubation, the slides were washed thoroughly with Optimax wash buffer (Biogenex, San Ramon, CA, USA). The immunoreaction was demonstrated using an HRP-based chromogen/substrate system including DAB (brown) chromogen (Liquid DAB Substrate Kit; Zymed, San Francisco, USA). The sections were then counterstained with Mayer’s haematoxylin, dehydrated in ascending alcohol concentrations, cleared with xylene, mounted and examined by light microscopy. The number of Hofbauer cells/per villous profile was calculated. Five villous profiles from each slide were evaluated at random in each DS and in each control specimen.

**Histomorphometric analysis**

Quantitative microscopic analysis was performed at ×400 magnification. A systemic grid with a square density of 100 squares/mm² was superimposed on the specimens. The number of p43-PLF-immunopositive Hofbauer cells within the measured field was determined. Five specimens of each study group were assessed at 17 and 20 weeks gestation and at least 20 fields of each case were screened. The assessment of the specimens was performed by a screener blinded to the genotype of the specimen. The data for the histomorphometric analysis are presented as mean ± SD. Student’s t-test was applied for comparison and P < 0.05 was considered significant.

The intensity of p43-PLF immunostaining in the fetal kidneys was assessed by a semiquantitative methodology. Five DS and five control kidney samples were used and four consecutive sections from each fetal kidney were analysed under ×200 magnification. The highest intensity was indicated by (+++).

**Results**

Immunohistochemical evaluation of normal placental tissue detected p43-PLF immunostaining in the Hofbauer cells as well as in the syncytial layer (Figure 1A–C). However, the expression varied according to gestational age. Strong immunostaining was localized in both the syncytial and Hofbauer cells in first trimester placental vili (Figure 1A, placenta at 8 weeks gestation), whereas it was localized predominantly in Hofbauer cells and only weakly in the syncytial layer at 15 weeks gestation (Figure 1B, placenta at 15 weeks gestation).

A difference in the immunostaining of p43-PLF between normal and DS placentae was observed at 17 weeks gestation. Only rare positively stained Hofbauer cells were detected in normal placentae while the syncytial layer was immunonegative for p43-PLF expression (Figure 1C). In comparison, DS placental tissue (Figure 1D) exhibited a significantly higher number of p43-PLF–positively stained Hofbauer cells (Table I). The number of immunopositive Hofbauer cells decreased in DS placentae only at 20 weeks gestation, 3 weeks later than in normal placentae (Figure 1E). No difference was observed in the immunostaining pattern of the syncytial layer of normal and DS placentae both at 17 and 20 weeks gestation (Figure 1C and D respectively), as the syncytial cells were negative for p43-PLF expression.

Evaluation of fetal kidneys was performed on specimens derived from normal (Figure 2A) and DS fetuses (Figure 2B) at 17 weeks gestation. Immunoreactivity was confined to the cells of the nephron’s proximal tubules in both normal and DS fetuses (Figure 2A,B). A difference in the intensity of p43-PLF immunostaining was observed at a similar gestational age: increased staining intensity was detected in the proximal tubules of DS kidneys compared with the intensity observed in the kidneys of normal fetuses (Table II).

**Discussion**

Enhanced expression of p43-PLF was observed in DS placentae and in fetal kidneys compared with normal controls. This phenomenon cannot be attributed to chromosome 21 trisomy since human ferritin sequences have not been identified on this chromosome (Garty et al., 1987).

The increased level of p43-PLF expression in the DS placentae as well as the delay in its down-regulation may reflect an over-expression of this protein. Such a pattern may indicate that some immunological impairment found among DS individuals (Ugazio et al., 1990; Hayes et al., 1997) starts early in the uterine life resulting later in abnormalities of the maturation of lymphocytes and their function (Murphy and Epstein, 1990; Garty et al., 1995). Similarly, high p43-PLF levels have also been found in those patients with immunosuppressive states, such as those suffering from various malignancies (Moroz et al., 1987; Rosen, 1996) or HIV infection (Moroz et al., 1989).

Alternatively, the difference in p43-PLF expression in DS placentae compared with normal placentae may result either from abnormal

### Table I. Histomorphometric quantification of the number of p43-placental Hofbauer cells per measured field (mean ± SD)

<table>
<thead>
<tr>
<th>Gestational age</th>
<th>Normal placenta</th>
<th>DS placenta</th>
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<tr>
<td>17 weeks</td>
<td>3.64 ± 2.9</td>
<td>9.68 ± 2.72</td>
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<tr>
<td>20 weeks</td>
<td>2.28 ± 1.6</td>
<td>4.05 ± 3.42</td>
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**Table II. Intensity of p43-placental isoferritin immunostaining in the fetal kidneys at 17 weeks gestation**

<table>
<thead>
<tr>
<th>Normal kidneys</th>
<th>DS kidneys</th>
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**Table III. Number of positive Hofbauer cells per measured field (mean ± SD)**

- *a n = 5 for each study group.*
- *b t-test; P < 0.005.*
placentation originating from a trisomic conceptus (Moroz et al., 2000) or from a primary developmental delay, as demonstrated in other haematopoietic systems of DS fetuses (Thilaganathan et al., 1995).

The increased maternal serum p43-PLF level that was measured in DS gestation (Moroz et al., 2000) may reflect the increased percentage of positive fetal Hofbauer cells crossing the feto-maternal interface. Similarly, increased numbers of other fetal cells have been detected in the maternal circulation in various cases of chromosomal aneuploidies (Elia et al., 1992; Simpson and Elias, 1993).

In the current investigation, we also found increased immunostaining of p43-PLF in DS fetal kidneys compared with normal controls. It is not known whether this immunoregulatory protein is produced by the fetal kidneys or, alternatively, is just retained there by an as yet unknown mechanism. In this respect, it was recently reported that glomerular epithelial cells from mouse kidneys produce CD2-associated protein (CD2AP) which interacts with CD2 (Shih et al., 1999). p43-PLF has been shown to exert immunosuppressive activity via binding to the CD2 antigen, which facilitates T cell adhesion to antigen-presenting cells (Moroz et al., 1997). It is possible that the binding of CD2AP to a human CD2 homologue in fetal kidneys is responsible for the retention of p43-PLF currently demonstrated.

Using the immunohistochemistry technique, we were able to confirm the over-expression of the recently cloned PLIF gene coding for p43, which has been proposed as a serum marker for DS screening (Moroz et al., 2000). The regulatory mechanism of p43-PLF expression is currently under investigation. It has already been shown to be over-expressed in breast cancer (Moroz et al., 2002), and may be associated with both the immuno-compromized state and the high malignancy rate characterizing DS individuals.

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References


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