The immunophenotypic profile of hepatic hemopoiesis in fetuses with Down's syndrome during the second trimester of development

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Summary

The yolk sac and aorto-gonad-mesonephros region are well recognized as the principal sites of hematopoiesis in the developing embryo, and the liver is the principal site of hematopoiesis in the fetus. In the present study, we investigated the immunohistochemical expression of Glycophorin C (erythrocytes), Neutrophilic elastase (granulocytes), and CD34 (progenitor hematopoietic stem cells, progenitor stromal cells, and vascular endothelial cells) in hepatic parenchyma from fetuses with Down's syndrome (DS) (16th, 20th, and 24th week of gestational age), and correlated the findings with the equivalent of the hepatic parenchyma from fetuses after spontaneous abortion.

Our results did not demonstrate a quantitative difference at the level of erythropoiesis in all three periods examined. In contrast, an important numerical difference was shown in the expression of CD34 positive cells in liver parenchyma from fetuses with DS, in comparison with those found in liver parenchyma from fetuses after spontaneous abortion ($p < 0.02$). Furthermore, a modest but significant difference was demonstrated at the level of granulopoiesis between the 20th and 24th week ($p < 0.01$). Given that, the living newborns with Down's syndrome manifest diverse haematological abnormalities, including a transient leukemoid reaction that usually disappears after some weeks or months, a significantly increased number of CD34 positive and a less significantly increased number of neutrophilic elastase positive cells between the 20th and 24th gestational week could explain this phenomenon in combination with the respective results, if any, in the bone marrow. Regarding our finding of increased stromal CD34 positive cells in the hepatic portal triads, it raises the possibility that a process similar to fibrosis of the bone marrow may contribute to the hepatic fibrosis in DS.

Key words: Liver hematopoiesis; Down's syndrome; Spontaneous abortion; Second trimester of gestation.

Introduction

Hematopoietic stem and progenitor cells reside in diverse anatomical locations in developing mammals including the yolk sac [1] and para-aortic region within the embryo [2-4] and the liver, spleen and bone marrow of the fetus [5-7]. The site of origin of definitive hematopoietic stem cells in the developing fetus remains controversial. Evidence from some studies indicates that hematopoietic stem cells from the yolk sac are responsible for transient primitive hematopoiesis, but they appear to lack the ability to reconstitute the hematopoietic system in adult animals [3, 8]. Instead, stem cells derived from an intraembryonic site, the aorta-gonad-mesonephros (AGM) region, have been shown, both in mice and man [4], to be responsible for definitive hematopoiesis [2, 3, 9] by first colonizing the fetal liver and later the bone marrow [10].

All types of hemopoietic cells derive from a small pool of immature uncommitted progenitor cells. During the past few decades the phenotype of the hemopoietic progenitors has been analyzed in detail. The first "precursor cell antigen" to be described was HPCA-1 (CD34) [11, 12]. This antigen is expressed on (almost) all types of hemopoietic progenitors. However, CD34 is also expressed on stromal cell progenitors and even on vascular endothelium [13-15].

Approximately 95% of all cases with Down's Syndrome (DS) harbor primary trisomy 21, about 4% harbor a translocation, and 1% a mosaic. The most frequent translocation is between chromosome 21 and a chromosome of the D (usually 14) or G (usually 22) groups. More than 50% of the D/G and 90% of the G/C translocations occur de novo, both parents having normal karyotypes. The remaining cases with D/G or G/C translocation have a parent with the same translocation in a balanced form; a balanced translocation can be found in several members of such families. The risk of inheriting D/G translocation in the mother is 11% and in the father 2.4%. In the very instance of (21q; 21q) translocation, the risk is 100% [16]. Infants with DS have intrauterine growth delay (IUGD). The phenotype of Down's syndrome can be recognized easily in the fetus as early as 14 weeks of gestation.

In midtrimester fetuses the principal site of hematopoiesis is the liver, where two functionally different compartments can be observed. Erythropoiesis mainly takes

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Fig. 1

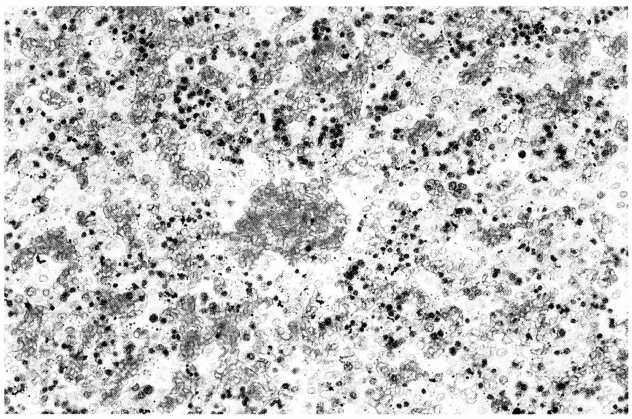


Fig. 2

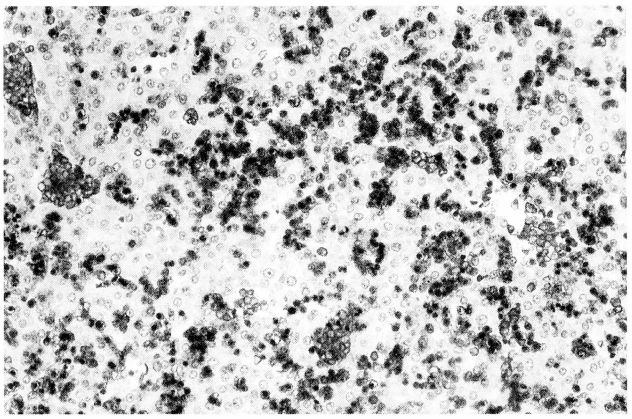
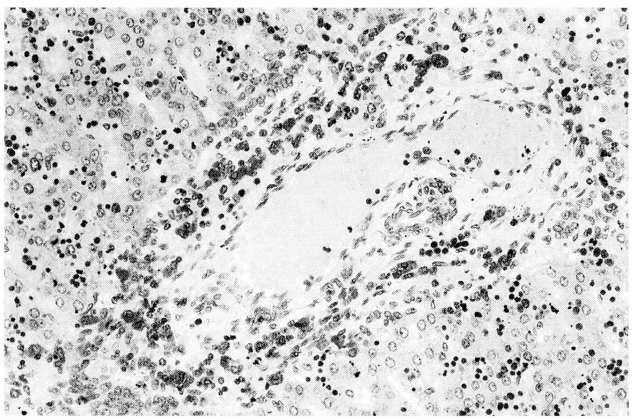


Fig. 3



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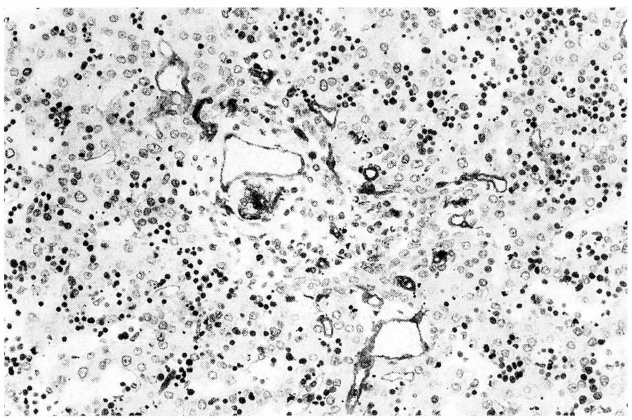

Fig. 5

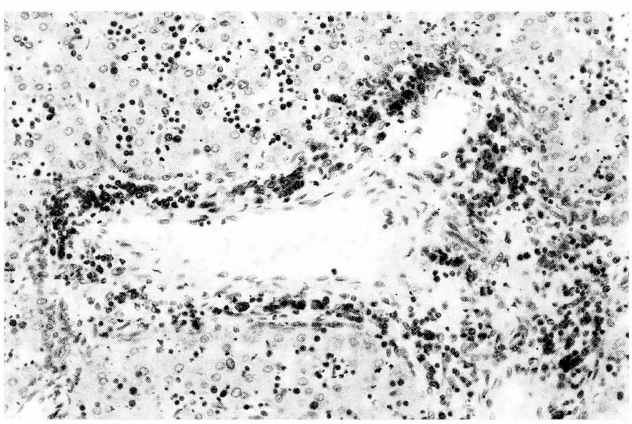

Fig. 6

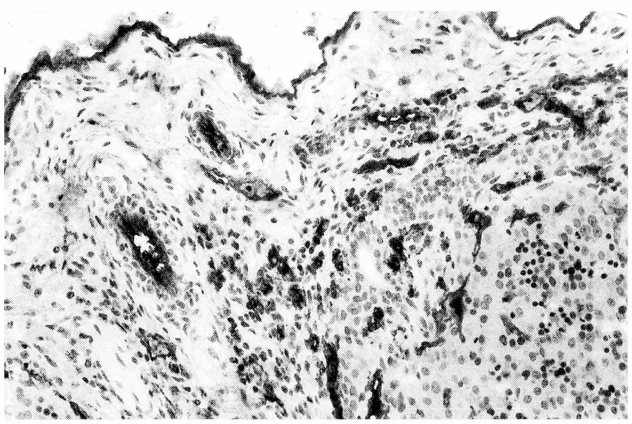

Figure 1. — Erythropoiesis occurring within the hepatic sinuses. Glycophorin C × 100 (spontaneous abortion).
Figure 2. — Erythropoiesis occurring within the hepatic sinuses. Glycophorin C × 100 (Down's syndrome).
Figure 3. — Granulopoiesis occurring within the mesenchymal tissue of the hepatic portal fields. Neutrophilic elastase × 100 (spontaneous abortion).
Figure 4. — Immunohistochemical control for CD34 showing an intense staining pattern in the progenitor hematopoietic stem cells, progenitor stromal cells and endothelial cells of the vessels × 100 (spontaneous abortion).
Figure 5. — Granulopoiesis occurring within the mesenchymal tissue of the hepatic portal fields. Neutrophilic elastase × 100 (Down's syndrome).
Figure 6. — Immunohistochemical control for CD34 showing a strong reactivity with the progenitor hematopoietic stem cells, progenitor stromal cells and endothelial cells of the vessels × 100 (Down's syndrome).

place within the sinusoids of the parenchyma while granulopoiesis is restricted to the mesenchymal tissue of the portal fields.

This study was performed to evaluate whether the immunohistochemical expression of Glycophorin C (erythrocytes), Neutrophilic elastase (granulocytes), and CD34 (progenitor hematopoietic stem cells, progenitor stromal cells, and vascular endothelial cells) in hepatic fetal parenchyma, during the second trimester of gestation, provide more arguments for the haematological disturbances that may occur during the first weeks of a living newborn with Down's syndrome.

Materials and Methods

Samples representing 15 tissue sections of the right lobe of the liver from fetuses with Down's syndrome and 15 tissue sections of the right lobe of the liver of fetuses from embryos after spontaneous abortion were obtained at the 16th, 20th and 24th week of gestation. Livers were cut as thick as 3 mm, then fixed in 10% neutral buffered formaldehyde at 4°C for 24 hours and processed for routine paraffin embedding. Paraffin blocks were available in all cases, and tissue sections were stained with hematoxylin-eosin (H-E), PAS, Giemsa and Gomori.

Immunohistochemistry: A panel of monoclonal antibodies, all provided by DAKO was applied; particularly, Glycophorin C for the identification of erythropoiesis, Neutrophilic elastase for granulopoiesis and CD34 for the identification of immature hematopoietic progenitors cells, stromal progenitors cells, and vascular endothelial cells.

Immunohistochemistry was performed using the "avidin-biotin-immunoperoxidase" technique [17]. Sections were deparaffinized and the endogenous peroxidase activity was blocked by methanol with 0.3% H_2O_2 (30 min, RT). First-step Ab (avidin-biotin) were diluted in TBS and 1% bovine serum albumin (BSA) and applied for 60 min (RT). In case of CD34 staining, sections were treated with 0.3% H_2O_2 instead of methanol. After each step, the tissue was rinsed twice in 0.05 M TBS. For individual Ab, the "antigenicity" was enhanced by antigen retrieval as described [18]. Isotype-matched Ab were used as negative controls. After incubation with first-step Ab, slides were washed in TBS and then incubated with appropriate second-step biotinylated Ab for 30 min (RT). Then, slides were washed and incubated with the avidin-biotin-complex for 30 min (RT) or with peroxidase-conjugated streptavidin. After washing, bound Ab were visualized by incubating in 3-amino-9-ethyl carbazole (AEC) for 5 to 10 min. Sections were subsequently counterstained with hematoxylin and cover-slipped.

Results

Five microscopic fields of the parenchyma of the liver were evaluated in each case without knowledge of the clinical data, and the number of stained cells per square millimeter was calculated. The sections were examined independently by two observers, and positive cellular staining for each antibody was manifested as fine red cytoplasmic granularity.

16th week of gestation: The livers of fetuses in both cases (Down's syndrome and those after spontaneous abortion) showed a distinct distribution of hematopoiesis; erythropoiesis mainly occurred within the sinuses (average 3,820 cells/mm^2, range 2,918 to 4,935 cells/mm^2) (Figures 1, 2), and granulopoiesis primarily within the mesenchymal stromal cells of the portal fields (average 32 granulopoietic cells/mm^2, range 12 to 75 cells/mm^2) (Figure 3). The immunohistochemical control for CD34 to identify the progenitor hematopoietic stem cells showed an intense staining pattern by endothelial cells of the vessels within the portal fields (average 45 CD positive cells/mm^2, range 18 to 75 cells/mm^2), while positive expression of CD34 was demonstrated in the stromal cells of the mesenchymal portal tissue (Figure 4). An inconspicuous number of sinusoids close to the portal triad showed immunohistochemical expression of CD34 by the endothelial cells.

20th week of gestation: In this period of gestation within the hepatic parenchyma in both cases, no significant quantitative increase concerning erythropoiesis and granulopoiesis in the sinuses and portal fields, respectively, was noted. In contrast, in our series of Down's syndrome, a slight increase in the number of CD34 positive endothelial cells of the vessels and in the stromal cells of the mesenchymal portal tissue was shown ($p < 0.02$) (average 66 CD positive cells/mm^2, range 48 to 97 cells/mm^2). In addition, a significant number of sinusoids demonstrated an intense expression of CD34 by the endothelial cells.

24th week of gestation: During this period there was no significant quantitative difference concerning erythropoiesis in either setting. In contrast, in our cases of Down's syndrome a much greater portion of the mesenchymal tissues of the portal triads demonstrated granulopoietic activity ($p < 0.01$) (the number of granulopoietic cells was more than 2 times higher than those found in spontaneous abortions, average 70 granulopoietic cells/mm^2, range 25 to 160 cells/mm^2) (Figure 5), while the quantitative expression of CD34 by endothelial of the vessels within the portal fields was as intense as in the stromal cells of the mesenchymal portal tissue ($p < 0.02$) (average 105 CD positive cells/mm^2, range 58 to180 cells/mm^2) and in endothelial cells of the extending sinusoids (Figure 6).

Discussion

The existence of a multipotent mesenchymal progenitor giving rise to both hematopoietic and non-hematopoietic cells has been postulated by several investigators [19-21]. However, whereas such a totipotent cell clearly is of biologic significance in an embryonal phase of tissue development, no definitive proof for the existence of such a cell in the haematological disturbances manifested by living newborns with Down's syndrome (including a transient reaction which usually disappears after some weeks), has ever been presented. The increase of stromal CD34 positive cells comprises an indication of early fibrosis of the connective tissue background of the hepatic portal triads (Figure 6), which is a hallmark in most cases of perinatal liver disease affecting patients with Down's syndrome. In addition to an increased incidence of leukemia, patients with DS are known to have a phenomenon referred to variously as "transient myeloproliferative disorder", "transient abnormal myelopoiesis", or "congenital leukemoid reaction", or "congenital leukemia" [22]. Current data suggest that this transient hematologic proliferation is acute myeloblastic leukemia with a high rate of spontaneous regression [23]. Indeed, acute myeloblastic leukemia may represent approximately 40% of all leukemias in DS, while it represents less than 1% of leukemias in normal children [23]. Unfortunately, the vast majority of reports of myeloproliferative disorders in DS patients do not provide any description of liver histopathology. To obtain more data on the potential hepatic effects of disorders of hematopoiesis, we

compared the degree of extramedullary hematopoiesis of the livers in fetuses with Down's syndrome and those after spontaneous abortion. Our results of hepatic hematopoiesis showed that:

1) The comparative study of the quantitative percentage of *erythropoiesis* (Glycophorin C) at the 16th, 20th, and 24th week of gestation, remained stable in both sets (fetuses with Down's syndrome and those after spontaneous abortion).

2) The comparative study of the quantitative percentage of *granulopoiesis* (Neutrophilic elastase) remained the same at the 16th and 20th week of gestation in both series. In contrast, in the 24th week of gestational age a twofold increase in the number of granulopoietic cells was observed in the liver parenhyma of the fetuses with Down's syndrome in comparison with those after spontaneous abortion ($p < 0.01$).

The comparative study of the quantitative percentage of *CD34* for the identification of progenitor hematopoietic cells, stromal progenitors cells, and vascular endothelial remained the same at the 16th week of gestation in both sets. In contrast, a gradual increase of positive cells to this antibody in the liver parenchyma of the fetuses with Down's syndrome in comparison with those after spontaneous abortion, from the 20th until the 24th week, was observed ($p < 0.02$). This was more evident within the mesenchymal tissue of the hepatic portal fields and in the endothelial cells of the extending sinusoids.

A causal relationship between ineffective myelocytopoiesis and fibrosis of the bone marrow had already been assumed from morphologic evidence before experimental proof was presented [24]. An ineffective myelocytopoiesis may lead to an excessive concentration of stromal myoid cells in bone marrow that in turn stimulates fibroblastic proliferation and results in myelofibrosis [24]. A similar association could exist between ineffective hematopoiesis and fibrosis in the hepatic parenchyma of neonatals with Down's syndrome. The increase of stromal CD34 positive cells in the connective tissue of the hepatic portal triads may contribute to this direction. Further investigation is necessary to establish this hypothesis.

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