

# Human decidual cells can express the Hodgkin's cell-associated antigen Ki-1 (CD30) in spontaneous abortions during the first trimester of gestation

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## Summary

CD30 (Ki-1) antigen has been considered to be expressed on hematopoietic cells including the ones of the recently described anaplastic large cell lymphoma (ALCL), the Reed-Sternberg (RS) cells of Hodgkin's disease and the scattered large parafollicular cells in normal lymphoid tissues. Since then, several reports have been published describing CD30 expression in non-hematopoietic and malignant cells, such as cultivated human macrophages, human decidual cells, histiocytic neoplastic cells, mesothelioma cells, embryonal carcinoma, and seminoma cells. In the present study, we investigated the immunohistochemical expression of CD30 antigen in 15 paraffin-embedded placentas from fetuses after spontaneous abortion in the first trimester of gestation (8<sup>th</sup>, 10<sup>th</sup>, and 12<sup>th</sup> week, respectively) using the monoclonal antibody Ber-H2. All the pregnant patients had been given hormonal medication to support gestation. In addition, a panel of monoclonal antibodies for the identification of leukocytes (CD45/LCA), B-lymphocytes (CD20/L-26), and T-lymphocytes (CD45RO/UCHL1) was performed. Our findings were correlated with those found in 15 placentas obtained from 15 fetuses at the same time, after therapeutic or voluntary abortions.

This study demonstrates that, 1) decidual endometrial stromal cells are able to express the CD30 (Ki-1) antigen, 2) the expression of CD30 in decidual cells is higher in cases of hormonal administration (to support gestation), than that found in normal gestation. In the former cases (hormonal support of gestation), a mild mononuclear infiltration of the decidua by UCHL1 (T marker) positive cells, accompanies the CD30 positive cells.

**Key words:** CD30 (Ki-1) antigen; Decidual cells; Spontaneous abortion; Voluntary or therapeutic abortion; First trimester of gestation.

## Introduction

CD30 (Ki-1) antigen was first recognized on Hodgkin's and Reed-Sternberg cells by Stein *et al.* [1] using a monoclonal antibody raised against the Hodgkin's disease-derived cell line L428. The antibody also reacts with occasional large parafollicular cells in non-neoplastic lymphoid tissue and it has been suggested that these cells might represent the physiologic counterpart of Reed-Sternberg cells. Subsequently Ki-1 antigen has been demonstrated in a variety of other lymphomas, of predominantly T cell lineage. In particular the antigen is strongly and consistently expressed in the recently described anaplastic large cell lymphoma (Ki-1 lymphoma), which includes many cases previously designated as malignant histiocytosis [2]. Ki-1 antigen could also be induced in normal B and T lymphocytes after mitogen and virus transformation, but was not detectable in any other cell types [2]. Stein's group succeeded later in raising a second monoclonal antibody (Ber-H2) with a similar specificity to Ki-1 antibody that recognized a different, formalin-resistant epitope of the Ki-1 antigen, enabling it to be used in routinely processed tissue sections [3]. At the Third Workshop and Conference on Human Leucocyte Differentiation Antigens (Oxford, September 1986) these

antibodies were clustered in the new CD30 group and were recognized as defining a lymphocyte (B or T) activation antigen [4].

As known from the findings by Ito and colleagues [5] that, 1) normal human endometrial decidual stromal cells express the CD30 (Ki-1) molecule, and 2) the number of cells expressing the various lymphocytic markers changes throughout pregnancy [6], we investigated placentas from fetuses after spontaneous abortion occurring in pregnant women receiving hormonal support during the first trimester of gestation and correlated our data with those observed in placentas from fetuses after therapeutic or voluntary abortion performed on an equal number of pregnant women at the same time.

## Materials and Methods

Samples representing 15 placentas from fetuses after spontaneous (involuntary) abortion occurring in pregnant women administered with progesterone (300-600 mg per os until the 12<sup>th</sup> gestational week) and 15 placentas from fetuses after therapeutic or voluntary abortion were obtained at the 8<sup>th</sup>, 10<sup>th</sup> and 12<sup>th</sup> week of gestation. No hydropic changes in the chorionic villi of the placental tissue were observed in our material. Placentas 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 3-µm thick tissue sections were stained routinely

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with hematoxylin-eosin, PAS and Giemsa, and subsequently, using immunohistochemistry. The immunoperoxidase method was performed as follows: sections were deparaffinized in 70% alcohol and endogenous peroxidase was blocked with 3% H<sub>2</sub>O<sub>2</sub> in methanol. Sections were preincubated in 20% serum of the species from which the secondary antibody was raised and the primary antibody was applied. After overnight incubation at room temperature, the secondary biotinylated antibody was applied for 30 minutes. Staining was visualized using the Vector Elite System (Vector Laboratories, Burlingame, CA) with diaminobenzidine as the chromogen. Sections were counterstained in diluted hematoxylin. The primary antibodies used were as follows: (CD30/Ber-H2) activated lymphoid cells, mouse monoclonal antibody (Dako), (CD45/LCA) leukocyte common antigen, mouse monoclonal antibody (Dako), (CD20/L-26) B-lymphocytes, mouse monoclonal antibody (Dako), and (CD45RO/UCHL1) T-lymphocytes, mouse monoclonal antibody (Dako).

*Analysis of CD30/Ber-H2 positive decidual cells:* For each sample, the CD30/Ber-H2 positive decidual population was assessed by enumeration of labeled cells in each tissue compartment for a minimum of five random fields per section viewed at 40-fold magnification through a grid. Cell number was calculated per 1 mm<sup>2</sup> of tissue section. The counted areas were selected from random placental tissue sections, taking into account that the ratio of the area of the decidual stroma according to the area of the chorionic villi was representative of the entire field. Areas with obvious necrosis or hemorrhage were excluded. Statistical analysis was undertaken using the ANOVA test.

## Results

Five microscopic fields of the placentas were evaluated in each case without knowledge of the clinical data. The sections were examined independently by two observers, and positive cellular staining for each antibody was manifested as fine red cytoplasmic granularity and/or surface membrane expression.

*8<sup>th</sup> week of gestation:* The immunohistochemical study of the placentas during this period for the detection of CD30/Ber-H2 positive cells, in cases of spontaneous (involuntary) abortions, showed small clusters or scattered, large-sized CD30/Ber-H2 positive decidual cells in all settings examined (Figures 1, 2), with percentages

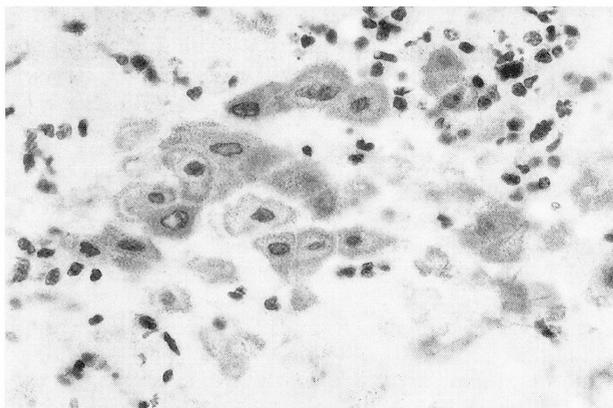


Figure 1. — Aggregates of large-sized CD30/Ber-H2 positive decidual cells (x400).

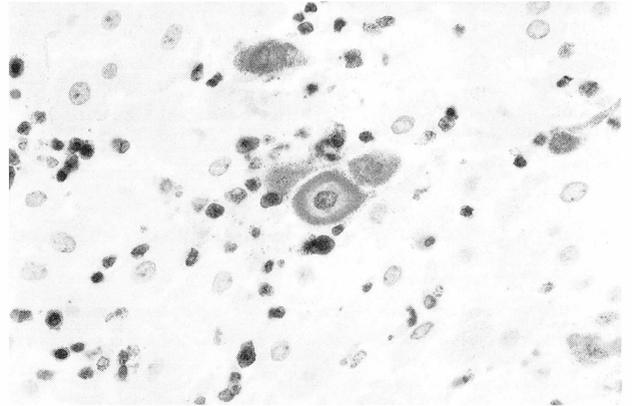


Figure 2. — Isolated large-sized CD30/Ber-H2 positive decidual cells (x400).

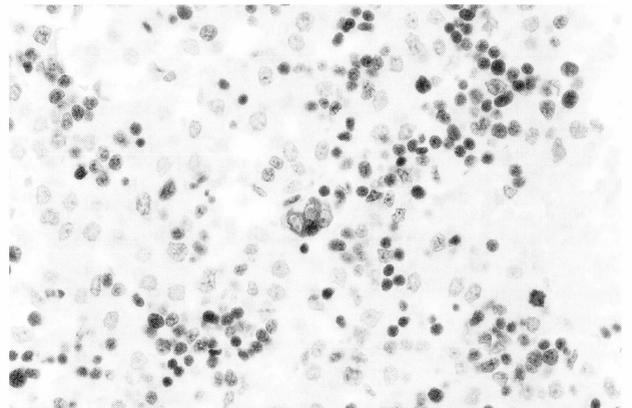


Figure 3. — Reed-Sternberg-like CD30/Ber-H2 positive cell showing bilobed nucleus, margined chromatin, eosinophilic inclusion-like nucleoli, and abundant cytoplasm against a background of lymphoplasmacytes (x400).

varying from 3.2 to 3.9 (mean values,  $3.61 \pm 0.16$ ). In the neighboring decidual stroma a slight cell infiltration was observed, consisting of rounded mononuclear cells of approximately 10  $\mu$ m in diameter with an eccentric kidney-shaped nucleus and expressing a CD45/LCA and CD45RO/UCHL1 phenotype. In one of our cases a dense lymphoplasmacytic infiltrate was observed, surrounding a large Reed-Sternberg-like cell with an intense nuclear membrane positivity to the CD30/Ber-H2 antigen (Figure 3). The microscopic examination (H-E, and PAS) of this R-S-like cell showed an abundant pale cytoplasm, a bilobed nucleus with margined chromatin, and prominent inclusion-like eosinophilic nucleoli. The immunohistochemical study of the placentas, in the cases of voluntary or therapeutic abortions, showed a smaller number of large-sized CD30/Ber-H2 positive decidual cells in all settings examined, with percentages varying from 3.1 to 3.7 (mean values,  $3.42 \pm 0.17$ ). No inflammatory infiltrates or necrosis were noted in the neighboring decidual stroma.

*10<sup>th</sup> week of gestation:* During this period, in cases of spontaneous abortions, the immunohistochemical exami-

nation for the identification of CD30/Ber-H2 decidual cells showed a higher number of positive cells in comparison with those found at the 8th week of gestation, with percentages varying from 4.9 to 5.6 (mean values,  $5.27 \pm 0.19$ ). The number of inflammatory infiltrates in the decidual stroma, expressing the phenotype CD45/LCA and CD45RO/UCHL1 was minimal. The immunohistochemical study of the placentas, in the cases of voluntary or therapeutic abortions, showed a relatively equal number of large-sized CD30/Ber-H2 positive decidual cells in comparison with those found at the 8th week of gestation, with percentages varying from 3.2 to 3.9 (mean values,  $3.43 \pm 0.18$ ). No inflammatory infiltrates or necrosis were noted in the neighboring decidual stroma.

*12th week of gestation:* During this time an even higher number of CD30/Ber-H2 positive decidual cells was found compared to that at the 10th week, with percentages varying from 4.8 to 5.7 (mean values,  $5.34 \pm 0.23$ ). Respectively, the number of CD30/Ber-H2 positive decidual cells in the cases of voluntary or therapeutic abortions was more or less the same as that at the 8th and 10th weeks, with percentages varying from 3.2 to 3.7 (mean values,  $3.41 \pm 0.17$ ). No differences in the immune reactions were noted in the neighboring decidual stroma in cases of spontaneous abortions as well as in cases of voluntary or therapeutic abortions in comparison to the 8th and 10th gestational weeks.

A statistically significant difference was found between CD30/Ber-H2 positive cells at the 8th, 10th, and 12th gestational week after spontaneous abortions ( $p < 0.0001$ ). No significant difference was observed between CD30/Ber-H2 positive cells at the 8th, 10th, and 12th gestational week after voluntary or therapeutic abortions ( $p = 0.95$ ).

## Discussion

Approximately 15% of all clinically recognized human pregnancies end in early spontaneous (involuntary) abortion (SAB) (7). Chromosomal disorders, the only well established etiology for early SABs, are found in approximately 50% of SABs [8-13]. Among cytogenetically abnormal SABs, there is a heterogeneous distribution of karyotypes, including trisomies (50% to 52%), monosomy X (15% to 22%), structural rearrangements (4% to 8%), and tetraploidy (2% to 7%) [10, 11]. Another type of chromosomal abnormality possibly important in SABs is confined placental mosaicism (CPM) in which the karyotype of the placenta differs from that of the embryo-fetus [14]. Although CPM may be increased in SABs [15], its relevance to early SABs remains unclear.

Recently, a suggestion was made that immunodependent miscarriages are triggered by abnormal response of T-Helper-1 cells (TH1) as far as Interferon- $\gamma$  (INF- $\gamma$ ) and tumor necrosis factor (TNF) production are concerned. Trophoblastic and embryonal progression is blocked due to activated lymphocytes and macrophages; both cell lineages activation is up-regulated by TNF and INF- $\gamma$ . In 70% of habitual abortions occurring in pregnancy, there was an abnormal response of TH1 cells compared to 3%

of abnormal response occurring in non-miscarrying women. Conclusively, cytokine production may induce reproduction processes in a direct or indirect fashion.

The embryo is a semi-allograft of a different nature, considering that it contains proteins coming from the father, and unfamiliar to the mother. There is a common sense, that circulating blocking factors protect embryos from maternal lymphocytes which may cause miscarriage due to reaction against paternal antigens. Such factors can be progesterone and chorionic gonadotropin; their levels in blood serum are low in cases of habitual abortions. Mixed lymphocyte culture has been applied to detect circulating blocking factors. However, the above hypothesis was not confirmed by well-developed perspective studies. In non-miscarrying women no circulating blocking factors were detected in serum to slow down mixed lymphocyte culture. Recently it has been implied that mixed lymphocyte culture results are representative of habitual abortion consequences rather than the causes of their appearance.

Our results support that CD30 expression in decidual stromal cells is induced by progesterone control. This would be a novel mechanism of CD30 induction other than neoplastic transformation and viral infection of lymphocytes. Studies of CD30 expressed in decidual cells would provide a new insight into its biological functions. The demonstration of the large Reed-Sternberg-like cells in the decidual stroma within a lymphoplasmacytic infiltrate, in the same way that similar R-S-like cells are observed in the reactive lymph nodes especially within the parafollicular areas, is evidence that such cells might represent the physiologic counterpart of true R-S cells.

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