OCCURRENCE OF CD30 ANTIGEN ON TISSUES AND CELLS OTHER THAN LYMPHOID ORIGIN. A STUDY OF HUMAN FETAL SKIN IN 8TH, 10TH, AND 12TH GESTATIONAL WEEKS

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Abstract. Objective**:** CD30 antigen has long been considered to be restricted to the tumour cells of Hodgkin's disease and of anaplastic large cell lymphoma as well as to T and B activated lymphocytes. Expression of CD30 antigen has been reported in the decidual stroma, cultivated macrophages, lipoblasts, myoepithelial cells, reactive and neoplastic vascular lesions, mesotheliomas, embryonal carcinoma and seminoma cells. The fact that the CD30 molecule can mediate signals for cell proliferation or apoptosis prompted us to perform a systematic investigation of CD30 antigen expression in embryonal tissues during proliferation and differentiation stages. We first targeted on the fetal human intestinal cryptae cells with positive results. The epidermis is a dynamic epithelium that is constantly renewed throughout life. Its turnover is estimated at about 7 days in mice and about 60 days in humans. This rapid replacement demands, as with other epithelial tissues, that an adult has stem cells capable of supplying differentiated cells throughout life. The most basic widely accepted criteria for these stem cells are that they have a high capacity of self-renewal and the ability to generate daughter cells that undergo terminal differentiation. The basal layer, attached to the basement membrane, contains the dividing cells of the skin and as cells move up from this layer they undergo differentiation, ending in the formation of a terminally differentiated anucleate cell called squame. Not all of the proliferative cells in the basal layer are stem cells. It is intriguing to find out if stem or other cells in the basal layer can express the CD30 antigen. Materials and methods**:** We investigated the immunohistochemical expression of CD30 antigen in 15 paraffin-embedded tissue samples representing epidermis and epidermal buds from fetuses after spontaneous abortion in 8th, 10th, and 12th week of gestation, respectively, using the monoclonal antibody Ki-1. Results**:** The results showed that: 1) the epithelial cells of the epidermis in the developing skin express the CD30 antigen; 2) CD30 expression in these epithelial cells is higher in cases of hormonal administration than in normal gestation; 3) A similar positive reaction involved the epidermal buds associated with the development of the skin appendages.

*Key words***:** CD30 antigen; human fetal skin; gestation.

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INTRODUCTION

The skin is the largest organ in the body. It consists of an outer layer, the epidermis, a stratified squamous epithelium derived from the ectoderm, and an inner layer, the dermis of mesodermal origin. The epidermis and dermis are separated by a basement membrane. The epidermis is made almost entirely of keratinocytes (95%). Other cell types found include melanocytes, Langerhans cells (dendritic cells), and Merkel cells (sensory receptors). During development the primitive epidermis arises as a single cell layer at the time when the ectoderm and endoderm are defined in the inner cell mass of the blastocysts. A second outer epidermal layer, the periderm, arises at the end of the first month in humans and by day 12 of embryonic development in mice [41]. A third intermediate layer forms between 4 and 9 weeks estimated gestational age in humans and days 13 and 16 in mice. Over the next few days in mouse development, the intermediate layer is replaced by strata spinosum and granulosum, and by day 17 the first cornified cells are observed. In human development, it takes 24 weeks for all the epidermal layers to form [16]. Mitotic activity in the early stages of development occurs in all layers [11], but as the suprabasal cells begin to display morphological signs of differentiation, mitotic activity becomes restricted to the cells in the cells of the basal layer.

The CD30 antigen is a 120 kD cytokine receptor which belongs to the tumour factor receptor (TNFR) superfamily [4, 8]. Initially, it was described as an antigen which is expressed on the surface of Reed-Sternberg and Hodgkin's disease (HD) and a few scatterd, mainly parafollicular, large, lymphoid cells in normal lymphoid tissues [34**,** 38]. The occurrence of CD30 on the tumour cells of anaplastic large cell lymphomas (ALCLs) defined this entity as a lymphoid malignancy [38]. The induction of CD30 expression in peripheral blood lymphocytes following mitogen stimulation or viral transformation established this glycoprotein as an activation molecule [10]. More recently, CD30 was shown to be expressed, together with other activation molecules, in the tumour cells of body cavity-based lymphomas [26]. Pallesen and Hamilton-Dutoir [27] were the first to report CD30 expression outside of the lymphoid tissue in 12 out of 14 cases of primary or metastatic embryonal carcinoma (EC) of the testis, by immunostaining with the monoclonal antibodies (MAbs) Ber-H2 and Ki-1. Subsequently, several investigators have confirmed their results and have detected CD30 in these carcinomas at the protein [7, 9, 20, 28] and the mRNA level [20]. Two reports demonstrated CD30 expression in 4/21 and 4/63 cases of testicular and mediastinal seminoma, and in the seminomatous components of 7/14 cases of mixed germ cell tumours of the testis, respectively [17**,** 39]. Suster et al. detected the CD30 antigen in 6/25 yolk sac tumours of the testis and mediastinum [39]. The expression of the CD30 antigen has also been reported in

other non-lymphoid tissues and cells, such as soft tissue tumours [23], decidual cells [18, 29], lipoblasts [37], myoepithelial cells [24], reactive and neoplastic vascular lesions [33], mesotheliomas [12], cultivated macrophages, and histiocytic malignancies [3].

We have so far been able to investigate only a single tissue from a small number of fetuses of early gestational age [40]. Pallesen and Hamilton-Dutoit [27] examined CD30 expression in normal adult, neonatal, and fetal (week 28) testes, as well as other tissues (brain, spinal cord, lung, gut, kidney, erythropoietic tissue, muscle, bone and connective tissue) from fetuses of 11 and 12 weeks gestational age, with negative results. This could be due to technical reasons. During the last decade, antigen retrieval on paraffin sections for immunohistology was improved by boiling instead of enzymatic digestion. Weak, non-reproducible immunohistological staining patterns of the CD30 MAb Ber-H2 generated by enzymatic digestion disappeared on applying this method.

We investigated CD30 expression in fetal human epithelial cells of the basal germinative layer in the epidermis and epidermal buds of the developing skin.

MATERIALS AND METHODS

Samples representing 15 skins from fetuses after spontaneous (involuntary) abortion occurring in pregnant women treated with progesterone (300–600mg per day until the 12th gestational week), and 15 skins from fetuses after therapeutic or voluntary abortion, were obtained in the 8th, 10th and 12th weeks of gestation. The Regional Ethics Committees approved the study. Written informed consent was obtained from all individuals and the procedures followed accorded with institutional guidelines. Skins were cut in 3mm slices and fixed in 10% neutral buffered formaldehyde at 4 ºC for 24 h, then processed for routine paraffin embedding. Paraffin blocks were available in all cases, and 3μm thick tissue sections were stained routinely with hematoxylin-eosin, PAS and Giemsa, and subsequently by immunohistochemistry. Immunoperoxidase labeling was performed as follows: sections were deparaffinized in 70% alcohol and endogenous peroxidase was blocked with 3% H_2O_2 in methanol. The 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 min. Staining was visualized with a Vector Elite System (Vector Laboratories, Burlingame, CA) using diaminobenzidine as the chromogen. The sections were counterstained with dilute hematoxylin. The primary antibodies used were as follows: (CD30/Ki-1) activated lymphoid cells, mouse monoclonal antibody (Novocastra); (CD45/LCA) leukocyte

common antigen, mouse monoclonal antibody (Dako); (CD20/L-26) B-lymphocytes, mouse monoclonal antibody (Dako); and (CD3) T-lymphocytes, mouse monoclonal antibody (Dako). We used the high temperature antigen unmasking technique for immunohistochemical demonstration of CD30/Ki-1 on paraffin sections (Novocastra). Control slides were incubated with nonimmunized rabbit serum. An anaplastic lymphoma case-slide (positive control) was run in parallel with the assay.

Analysis of CD30/Ki-1 positive epithermal cells: For each sample, the CD30/Ki-1 positive 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 numbers were calculated per mm^2 of tissue section. The counted areas were selected from random tissue sections, taking into account that the ratio of the area of the epidermal or/and bud stroma (*lamina propria*) to the area of surface epithelium was representative of the entire field. Areas with obvious necrosis or haemorrhages were excluded. Statistical analysis was performed using the ANOVA test.

RESULTS

Five microscopic fields of the skin were evaluated in each case without knowledge of the clinical data (Table 1). Two observers examined the sections independently, and positive cellular staining for CD45, CD20, and CD30/Ki-1 antibodies was manifested as fine brown cytoplasmic granularity and/or surface membrane expression. On the contrary CD30/Ki-1 expression was clearly nuclear.

Table 1

Expresion of CD30 antigen in epidermal and bud cells during the first trimester of gestation.

Spontaneous abortions				Voluntary abortions			
8th week	10th week	12th week	statistics	8th week	10th week	12th week	statistics
CD30(+)cells/mm^2				CD30(+)cells/mm^2			
3.58±0,13	5.24±0.16	5.31±0.20	$p < 0.0001$	3.39±0.14	3.40±0.15	3.38±0.14	$p = 0.92$

8th week of gestation: In cases of spontaneous (involuntary) abortion, immunohistochemistry revealed small clusters or scattered, large-sized CD30/Ki-1 positive epidermal and bud cells within the skin in all settings examined (Fig. 1), with percentages varying from 2.9 to 3.6 (mean ± SD = 3.58 ± 0.13). In the neighboring dermal stroma slight cellular infiltration was observed, consisting of rounded mononuclear cells approximately 10μm in diameter with eccentric kidney-shaped nuclei and expressing a CD45/LCA and CD3 phenotype. In cases of voluntary or therapeutic abortion, immunohistochemistry showed a smaller number

of large-sized CD30/Ki-1 positive epidermal and bud cells in all settings examined, with percentages varying from 2.8 to 3.4 (mean ± SD = 3.39 ± 0.14). No inflammatory infiltrates or necrosis were noted in the neighboring dermal stroma.

10th week of gestation: In cases of spontaneous abortion, immunohistochemistry showed a higher number of positive CD30/Ki-1 epidermal and bud cells than at the 8th week of gestation (Fig. 2), with percentages varying from 4.6 to 5.3 (mean ± SD = 5.24 ± 0.16). There were very few inflammatory infiltrates in the dermal stroma expressing the phenotype CD45/LCA and CD3. In cases of voluntary or therapeutic abortion, the frequency of CD30/Ki-1 positive epidermal and bud cells was similar to that at the 8th week of gestation, with percentages varying from 2.9 to 3.6 (mean ± SD = 3.40 ± 0.15). No inflammatory infiltrates or necrosis were noted in the neighboring dermal stroma.

12th week of gestation: In spontaneous abortion cases the number of CD30/Ki-1 positive epidermal and bud cells was even higher than at 10th week, with percentages varying from 4.5 to 5.4 (mean ± SD = 5.31 ± 0.20) (Fig. 3). The number in cases of voluntary or therapeutic abortions was more or less the same as at 8th and 10th weeks, with percentages varying from 2.9 to 3.4 (mean ± SD = 3.38 ± 0.14). No differences in immune reaction were noted in the neighboring dermal stroma in cases of either spontaneous or voluntary/therapeutic abortion in comparison to the 8th and 10th gestational weeks.

The differences among the numbers of CD30/Ki-1 positive cells at the 8th, 10th and 12th gestational week after spontaneous abortion were statistically significant ($p < 0.0001$). No significant differences were observed in the numbers of these cells after voluntary or therapeutic abortions ($p = 0.92$).

DISCUSSION

The value of the CD30 antigen as a diagnostic marker for Hodgkin's disease and anaplastic large cell lymphoma is well documented [34**,** 36**,** 39]. However, the function of this cytokine receptor in Hodgkin's disease and other CD30-positive diseases is still not clear. CD30 is preferentially expressed by activated lymphoid cells. In normal peripheral organs, however, CD30 expression is rather low. Resting peripheral blood lymphocytes were found to be negative for CD30. However, one recently published article showed that a variable proportion (3–31%) of circulating T cells in the normal peripheral blood are CD30+, and many of these are CD8+ T cells [2]. This variability in results is probably due to the sensitivity of the staining technique. CD30+ cells can also be detected within the parafollicular areas and in the rim of the follicular centers in the lymph nodes [13]. In addition, CD30+ cells are found in the medulla of the thymus, mainly around Hassal’s corpuscles [32]. B

cells also express CD30 to a variable extent, as do activated NK cells, endothelial cells, and decidual cells [6, 18, 32**,** 33, 35]. CD30 soluble form (sCD30) levels in normal individuals vary, but are usually very low [15**,** 19**,** 25**,** 31]. However, in some studies in which healthy blood donors were used as controls, very high sCD30 levels have been reported [5**,** 21], most notably in the younger age groups [21]. Since CD30 is upregulated after virus infections, the high sCD30 levels in these individuals could be explained by EBV infection [1].

In vitro studies have shown CD30 ligation can mediate a variety of signals, depending on cell type and origin, including enhanced cell growth or cell death of CD30+ cells [14**,** 22]. Cells with fetal origin, e.g. yolk sac carcinoma cells, have been shown to express CD30L [30] while CD30L expression in the placenta has not been reported.

Our results give the first indication that the CD30 antigen is expressed in the epithelial cells of the epidermis and epithermal buds of the developing skin. This observation has a number of important implications: First, our findings are of significance with regard to the supported origin of R-S cells. Care must be taken when drawing histogenetic conclusions based on the identification of a single marker in different cell types. Shared expression of CD30 antigen does not necessarily relate Hodgkin and R-S cells to activated lymphocytes. The identification of this antigen in cells as apparently disparate as activated lymphocytes, R-S cells and now human epithelial cells of the developing fetal skin suggests that previous theories as to the nature of the CD30 antigen must be re-examined. Although expression of CD30 antigen may indicate a relationship between these cell types, it is likely to be less straightforward than was previously supposed. Identification of the normal physiologic role of CD30 antigen is thus made even more imperative if these relationships are to be understood.

Second, these findings indicate that outside the lymphatic system, CD30 antigen expression in the epithelial cells of the epidermis and epidermal buds of the developing skin, can mediate signals for cell proliferation and differentiation in a region where other different types of cells (melanocytes, Langerhans' cells, Merkel cells) are growing all lifetime long.

CD30 also appears to be expressed in a selected group of terminally differentiated cells, which are responsive to hormonal stimulation. This variation of expression suggests a possible role for hormones, preferably progesterone, in the regulation of CD30 expression.

This is the first report that demonstrates CD30 in epithelial cells in fetal skin tissue. Although this must be confirmed in frozen section before it can be relied on, this finding together with reported positive staining seen in placenta [18**,** 29] suggests that the antigen is expressed by epithelial proliferating and differentiating cells of other than lymphoid origin. Clearly the extent of expression of CD30 antigen in embryonal tissues warrants further investigation.

The results of the present study provide additional evidence for a role of CD30 expression by epidermal cells in the epidermis and epidermal buds in the outcome of differentiation and events in the development of the skin.

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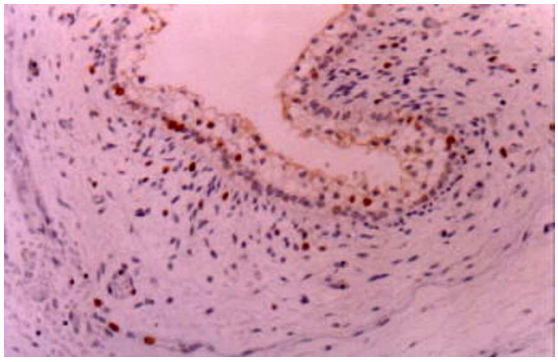

Fig. 1. 8th week of gestation (involuntary abortions). Ki-1 (CD30) antigen is expressed by epithelial epidermal cells. Immunohistochemical stain X 100.

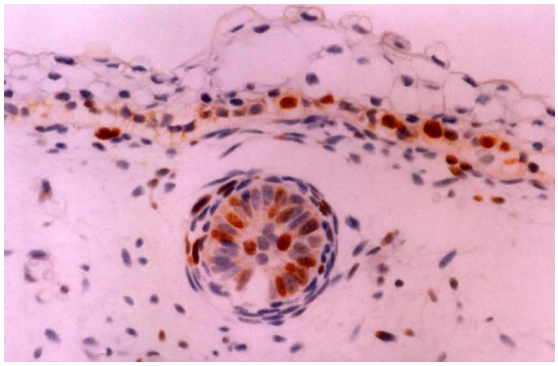

Fig. 2. 10th week of gestation (involuntary abortions). Expression of Ki-1 (CD30) antigen in the developing epidermis. Immunohistochemical stain X 200.

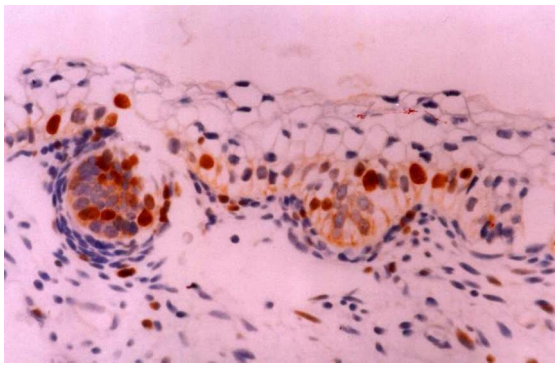

Fig. 3. 12th week of gestation (involuntary abortions). Expression of Ki-1 (CD30) antigen in the developing epidermis. Immunohistochemical stain X 200.