CD30 (Ki-1) molecule expression in human embryonal epithelial cells of the basal layer of the developing epidermis and epidermal buds and its potential significance for embryogenesis.

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ABSTRACT

Objective: CD30 antigen has long been considered to be restricted to tumour cells of Hodgkin's disease, of anaplastic large cell lymphoma and 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 the proliferation and differentiation stages. We first targeted foetal 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 and widely accepted criteria for these stem cells are that they have a high capacity for self-renewal and the ability to generate daughter cells that undergo terminal differentiation. Not all of the proliferative cells in the basal layer are stem cells and we were intrigued 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 foetuses after spontaneous abortion in 8th, 10th, and 12th week of gestation, respectively, using the monoclonal antibody Ki-1.

Results: The results showed that the epithelial cells of the epidermis in the developing skin express the CD30 antigen and CD30 expression in these epithelial cells is higher in cases of hormonal administration than in normal gestation. A similar positive reaction was observed in the epidermal buds associated with the development of the skin appendages.

KEY WORDS

CD30 antigen, human foetal skin, gestation

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 (1). A third intermediate layer forms between 4 and 9 weeks of the estimated gestational age in humans and between 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 can be observed. In human development, it takes 24 weeks for all the epidermal layers to form (2). Mitotic activity in the early stages of development occurs in all layers (3), but as the suprabasal cells begin to display morphological signs of differentiation, mitotic activity becomes restricted to the cells of the basal layer.

The CD30 antigen is a 120 kD cytokine receptor which belongs to the tumour factor receptor (TNFR) superfamily (4,5). Initially, it was described as an antigen which is expressed on the surface of cells in Reed-Sternberg (RS) and Hodgkin's disease (HD) and on a few scattered, mainly parafollicular, large lymphoid cells in normal lymphoid tissues (6,7). The occurrence of CD30 in the tumour cells of anaplastic large cell lymphomas (ALCLs) defined this entity as a lymphoid malignancy (7). The induction of CD30 expression in peripheral blood lymphocytes following mitogen stimulation or viral transformation established this glycoprotein as an activation molecule (8). More recently, CD30 was shown to be expressed, together with other activation molecules, in the tumour cells of body cavity-based lymphomas (9). Pallesen and Hamilton-Dutoir (10) reported CD30 expression outside the lymphoid tissue in 12 out of 14 cases of primary metastatic germ-cell tumours of the testis by immunostaining with the monoclonal antibodies Ber-H2 and Ki-1. Subsequently, several investigators have confirmed their results and have detected CD30 in these carcinomas at the protein (11-14) and the mRNA level (14). 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 (15,16). Suster et al. detected the CD30 antigen in 6/25 yolk sac tumours of the testis and mediastinum (16). The expression of the CD30 antigen has also been reported in other non-lymphoid tissues and cells, such as soft tissue tumours (17), decidual cells (18,19), lipoblasts (20), myoepithelial cells (21), reactive and neoplastic vascular lesions (22), mesotheliomas (23), cultivated macrophages, and histiocytic malignancies (24).

We have so far been able to investigate only a single tissue from a small number of foetuses of early gestational age (25).

Pallesen and Hamilton-Dutoit (10) examined CD30 expression in normal adult, neonatal, and foetal (week 28) testes, as well as in other tissues (brain, spinal cord, lung, gut, kidney, erythropoietic tissue, muscle, bone and connective tissue) from foetuses of 11 and 12 weeks of gestational age, with negative results. This could be due to technical reasons. During the last decade however, antigen retrieval on paraffin sections for immunohistology has been 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.

Materials and methods

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

15 skin samples from foetuses after spontaneous (involuntary) abortion occurring in pregnant women treated with progesterone (300-600mg per day until the 12th gestational week), and 15 skin samples from foetuses after therapeutic or voluntary abortion, were obtained in the 8th, 10th and 12th weeks of gestation. The study was approved by Regional Ethics Committees, written informed consent was obtained from all individuals and the procedures followed accorded with institutional guidelines. The skin was cut into 3mm slices and fixed in 10% neutral buffered formaldehyde at 4°C for 24 h, then processed for routine paraffin embedding. Paraffin blocks were available in all cases, and 3 μm 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 minutes. Staining was visualized with a Vector Elite System (Vector Laboratories, Burlingame, CA) using diaminobenzidine as the chromogen. The sections were counterstained with diluted haematoxylin. 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,

Table1. Expression of the CD30 (Ki-1) antigen in epidermal and bud cells of foetal skin during the first trimester of gestation. Number of CD30 cells/mm² of tissue section.

week of gestation	spontaneous abortion	voluntary abortion
8th week	3.58 ± 0.13 (2.9 - 3.6)	3.39 ± 0.14 (2.8 - 3.4)
10th week	5.24 ± 0.16 (4.6 - 5.3)	3.40 ± 0.15 (2.9 - 3.6)
12th week	5.31 ± 2.20 (4.5 - 5.4) p>0.92	3.38 ± 0.14 (2.9 - 3.4) p<0.0001

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 nonimmunised rabbit serum. An anaplastic lymphoma case-slide (positive control) was run in parallel with the assay.

Analysis of CD30/Ki-1 positive epidermal cells: for each sample, the CD30/Ki-1 positive population was assessed by counting the labelled cells in each tissue compartment from a minimum of five random fields per section viewed at 40-fold magnification through a grid. Cell numbers were calculated per mm² 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 haemorrhage were excluded. Statistical analysis was performed using the ANOVA test.

Results

Five microscopic fields of the skin sections were evaluated in each case without knowledge of the clinical data (Table 1). Two observers examined the sections independently, and positive CD30 staining was manifested as a fine brown nuclear expression, a thus far unreported location of the antigen.

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 neighbouring dermal stroma slight cellular infiltration was observed, consisting of rounded mononuclear cells approximately 10 μm 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 neighbouring 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 neighbouring 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 the 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 the 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 neighbouring 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 (26-28). However, the

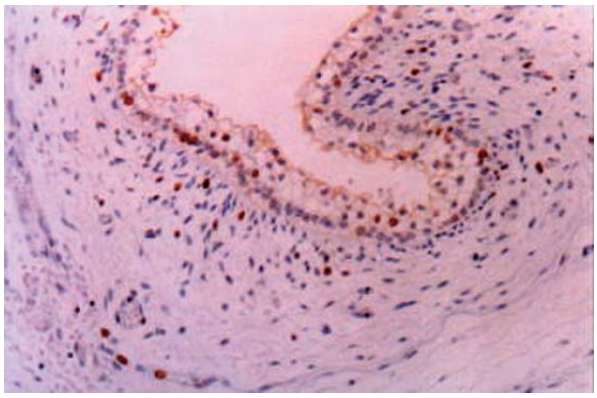

Figure 1. 8th week of gestation (involuntary abortions). Ki-1 (CD30) antigen is expressed by epithelial epidermal cells. Immunohistochemical stain X 100.

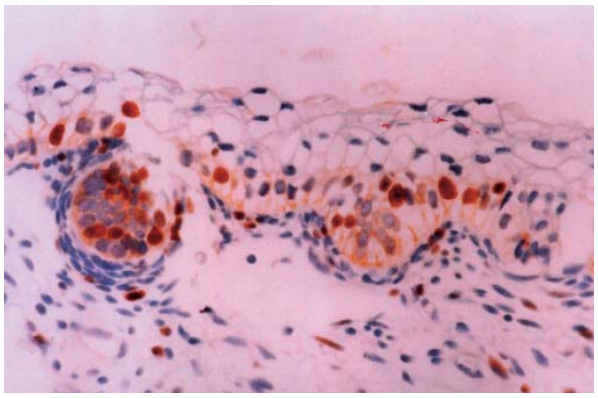

Figure 2. 10th week of gestation (involuntary abortions). Expression of Ki-1 (CD30) antigen in the developing epidermis. Immunohistochemical stain X 200.

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 normal peripheral blood are CD30+, and many of these are CD8+ T cells (29). 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 centres in the lymph nodes (30). In addition, CD30+ cells are to be found in the medulla of the thymus, mainly around Hassal's corpuscles (31). B cells also express CD30 to a variable extent, as do activated NK cells, endothelial cells, and decidual cells (31-35). sCD30 levels in normal individuals vary, but are usually very low (35-38). However, in some studies in which healthy blood donors were used as controls, very high sCD30 levels have been reported (39,40), most notably in the younger age groups (40). Since CD30 is up regulated after virus infections, the high CD30 levels in these individuals could be explained by EBV infection (41).

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 (42,43). Cells with foetal origin, such as yolk sac carcinoma cells, have been shown to express CD30L (44), whereas CD30L expression in placenta has not been reported.

Our results give the first indication that the CD30 antigen is expressed in epithelial cells of the epidermis and epidermal buds of the developing skin. This observation has a number of important implications: first, our findings are of significance with regard to the supposed 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 the CD30 antigen does not necessarily relate Hodgkin and R-S cells to activated lymphocytes. The identification of this antigen in cells so diverse as activated lymphocytes, R-S cells and now human epithelial cells of the developing foetal 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. It is of the utmost importance to identify the normal physiological role of the CD30 antigen 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

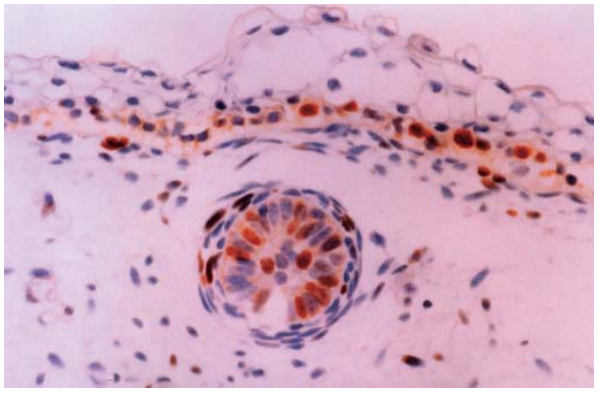

Figure 3. 12th week of gestation (involuntary abortions). Expression of Ki-1 (CD30) antigen in the developing epidermis. Immunohistochemical stain X 200.

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 throughout a normal lifetime.

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 foetal skin tissue. Although it must be confirmed in frozen section before it can be relied on, this finding, taken together with the reported positive staining seen in placenta (18,19) suggests that the antigen is expressed by epithelial proliferating and differentiating cells whose origin is not lymphoid. Clearly the extent of CD30 antigen expression in embryonal tissues warrants further investigation.

The results of the present study provide additional evidence of the role of CD30 expression in epidermal cells, in both the epidermis and epidermal buds, and its influence over the outcome of differentiation and other events in the development of the skin.

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