Human Embryonal Tissues of all Three Germ Layers can Express the CD30 Antigen. An Immunohistochemical Study of 30 Fetuses Coming after Therapeutic Abortions from Week 8th to week 16th of Gestation

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Summary

Originally, expression of the CD30 antigen was shown to be typical of the tumor cells of Hodgkin disease and of anaplastic large cell lymphomas. In reactive lymphoid tissue, CD30 is expressed only in a small population of activated lymphoid blasts. Since then, several reports have been published describing CD30 expression in non lymphoid tissues and neoplasms, such as embryonal carcinomas, seminomas, cultivated macrophages, histiocytic neoplastic cells, deciduals cells, and mesothelioma cells. In order to gain insight into the functions of CD30, given that it can mediate signals for cell proliferation and apoptosis, we studied the distribution of the antigen in different fetal archival paraffin-embedded tissues from week 8th to 16th of gestation.
We investigated the immunohistochemical expression of CD30 in 30 paraffin-embedded tissue samples representing all three germ layers, using the monoclonal antibody Ber-H2
CD30 is expressed early in human fetal development (8th–10th week) in a wide variety of tissues, with the exception of the skin and thymus in which it is expressed later on. This is consistent with the observation that these organs are not fully differentiated before 10th and 13th week, respectively. No expression was observed in the cardiovascular and respiratory systems.
The finding of CD30 expression in the terminal period of organogenesis, period, which is highly hormone related, implies that the antigen has an important role in cell development, maturation, and pathway to terminal differentiation in almost all fetal tissues and structures.

Key words: CD30 antigen – fetal tissues – 8th–16th week of gestation

Souhrn

Lidské embryonální tkáně všech tří zárodečných listů mohou exprimovat CD30 antigen. Imunohistochemická studie 30 plodů z léčebných potratů v 8.–16. týdnu gestace

Exprese antigenu CO30 byla považována za typickou pro nádorové buňky Hodgkinovy nemoci a anaplastického velkobuněčného lymfomu. V reaktivní lymfatické tkáni je CD30 exprimován pouze aktivovanými lymfoblasty. V poslední době se však množí zprávy o expresi CD30 nelymfatickými tkáněmi a nádory, jako jsou embryonální karcinom, seminom, kultivované makrofágy, nádorové histiocty, buňky deciduy, a buňky mezoteliomu. Vzhledem ke skutečnosti, že CD30 může zprostředkovávat signály pro buněčnou proliferaci a apoptózu, sledovali jsme pomocí monoklonální protilátky Ber-H2 distribuci tohoto antigenu v archivních parafinových bločcích tkání všech tří zárodečných listů plodů stáří 8.–16. týdnu gestace.
CD30 je exprimován již v časném fetálním období (8.–10. týdnu) v nejrůznějších tkáních orgány, s výjimkou kůže a thymu, v nichž dochází k expresi později. To odpovídá skutečnosti, že tyto nejsou

před 10., resp. 13. týdnem ještě diferencovány. V kardiovaskulárním a dýchacím systému jsme expresi neprokázali.
Závěr: Průkaz exprese CD30 v období končící organogeneze, které je výrazně hormonálně závislé, ukazuje, že tento antigen hraje důležitou roli v buněčném vývoji, vyzrávání a přechodu k plné diferenciaci téměř všech fetálních tkání a struktur.

Klíčová slova: CD30 antigen – fetální tkáně – 8.–16. týden gestace

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The CD30 antigen is a 120 kD cytokine receptor which belongs to the tumour necrosis factor receptor (TNFR) superfamily (1, 2). Currently, it is known that the expression of CD30 antigen is not limited to lymphoid tissue and lymphoproliferative disorders. For instance, strong CD30 expression has been documented in embryonal carcinoma and human decidual cells (3-5). Cytoplasmic Ber-H2 immunohistochemical staining has been reported in occasional carcinomas, many sarcomas, and vascular tumours (3). In addition, Ber-H2 immunohistochemical staining can be demonstrated in pancreatic tissue (3), some salivary gland tumours, and normal salivary gland tissue (3). The fact that the CD30 molecule can mediate signals for cell proliferation or apoptosis (6) prompted us to perform a systematic investigation of the antigen distribution in embryonal tissues using immunohistochemistry, from week 8^{th} onwards, in an effort to uncover patterns of expression that may elucidate the potential role of the marker during development stages.

MATERIALS AND METHODS

Tissue procurement

The tissue material (30 fetuses) used in this study was obtained from the files of the Department of Histology – Embryology at the University of Thrace. Samples representing a wide variety of tissues from all systems were collected from 30 fetuses: 15 males and 15 females (15 at 8^{th} to 10^{th} week of gestation and 15 at 12^{th} to 16^{th}, respectively) after therapeutic abortion. The organs used did not show any evidence of morphological abnormality. The Regional Ethics Committees approved the study. Written informed consent was obtained from all individuals and the procedures followed accorded with institutional guidelines.

Sections of tissue roughly 3-mm thick were fixed in 10% buffered formaldehyde for 7 hours then subjected to routine processing and paraffin embedding. Slides were obtained in all cases, and stained with hematoxylin-eosin (H-E), PAS, Giemsa and Gomori for morphological evaluation.

Monoclonal antibody and immunohistochemical staining

Antigen retrieval from formalin-fixed, paraffin-embedded tissue was performed by heating unstained sections immersed in DAKO Target Retrieval Solution (DAKO, Carpintera, CA) according to the manufacturer's instructions. A modified labeled avidin-biotin immunohistochemical staining was performed with the use of the LSAB-2 System Peroxidase Kit (DAKO) on DAKO Autostainer, according to the manufacturer's instructions. In short, deparaffinized sections were incubated with 3% hydrogen peroxide for 5 min., followed by 10-min. incubation with 1:20 solution of Ber-H2 MAb (Novocastra Laboratories Ltd., Newcastle, UK). That was followed by sequential 10-min. incubations with a biotinylated link antibody and peroxidase-labeled streptavidin. Staining was completed after a 10-min. incubation with DAKO Liquid 3,3'-diaminobenzidine Substrate-Chromogen System utilizing 3,3'-diaminobenzidine (DAB) chromogen. Biopsied lymph nodes of Hodgkin's disease were used as controls. All cases were coded, and the grading of the immunostaining was performed on a sliding scale of 1+ to 4+ according to the percentage of reactive cells (0 = no staining, 1+ = 1% to 25%; 2+ = 26% to 50%; 3+ = 51% to 75%; 4+ > 75%) Staining intensity was not the same in each one case. The scores represent the immunostained cells observed in the majority of cases.

RESULTS

Five microscopic fields of each tissue were evaluated in each case. The results of the immunostaining are summarized in table 1. Two observers examined the sections independently, and positive cellular staining was manifested as

fine brown cytoplasmic granularity and/or surface membrane expression. Immunostaining of tissue sections revealed the presence of CD30 antigen in many tissue types in variable abundance, mainly during the first period (8^{th} to 10^{th} week of gestation). Especially, in the gastrointestinal tract, positive staining for CD30 was observed in the epithelial cells (Fig. 1) of the developing primitive crypts (epithelial down-growths into the mesenchyme between the villi) of the small intestine. For the special glands of the postpharyngeal foregut, a weak cytoplasmic expression of CD30 was noticed in hepatocytes sparing the haematopoietic cells (Fig. 2), whereas

Tab. 1: Expression of CD30 by fetal tissues

		Gestational age (weeks)			
		8-10	12 -16	8-10	12 -16
Organ/tissue	Cell type	No. of positive cases		Staining intensity	
Gastrointestinal system					
Small intestine	Epithelium	10/15	0/15	2+	0
Liver	Hepatocytes	8/15	0/15	3+	0
Pancreas	Duct epithelium	12/15	0/15	3+	0
	Acini	12/15	0/15	3+	0
Urinary system					
Kidney	Tubular epithelium	12/15	0/15	3+	0
Renal pelvis	Epithelium	12/15	0/15	3+	0
Musculoskeletal system					
Long bones (endochondral ossification)	Perichondrium	9/15	0/15	1+	0
Flat bones (mesenchymal ossification)	Primitive mesenchymal cells	8/15	0/15	1+	0
Reproductive system					
Ovary (cortex)	Stromal cells	10/15	0/15	1+	0
Testis (medulla)	Stromal cells	5/15	0/15	1+	0
Nervous system					
Cerebral cortex	Neurons	13/15	0/15	3+	0
Cerebellum	Purkinje cells	11/15	0/15	2+	0
Endocrine system					
Adrenal gland	Cortex	0/15	0/15	0	0
	Medulla	15/15	0/15	4+	0
Hematolymphoid system					
Thymus medulla	Hassal's corpuscles	15/15	15/15	4+	4+
	Epithelial cells	15/15	15/15	2+	2+
cortex	Epithelial cells	15/15	15/15	2+	2+
Skin					
Epidermis	Basal cells	15/15	0/15	2+	0
Adnexa	Basal cells (epidermal buds)	15/15	15/15	2+	2+
Respiratory system	Epithelium	0/15	0/15	0	0
	Mesenchyme	0/15	0/15	0	0
Cardiovascular system		0/15	0/15	0	0

a strong positivity was observed in the epithelial cells of the pancreatic ducts and acini. In the urinary system, CD30 was expressed in the epithelial cells of the tubules and collecting ducts in the cortex of the kidney (Fig. 3), and in the epithelial cells lining the pelvis. In the musculoskeletal system (long bones), during the process of intracartilagineous (endochondral) ossification, positive CD30 cells were noted in the condensation of mesenchymal cells that constituted the perichondrium of the cartilagenous masses, at the site of the primary ossification centers (Fig. 4). In the intramembranous (mesenchymal) ossification (flat bones), positive

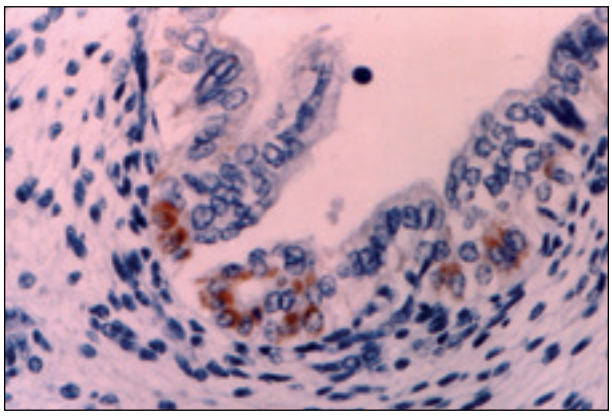

Fig. 1. Small intestine: CD30 expression in the epithelial cells at the bottom of the developing primitive crypts. Immunostain x 200

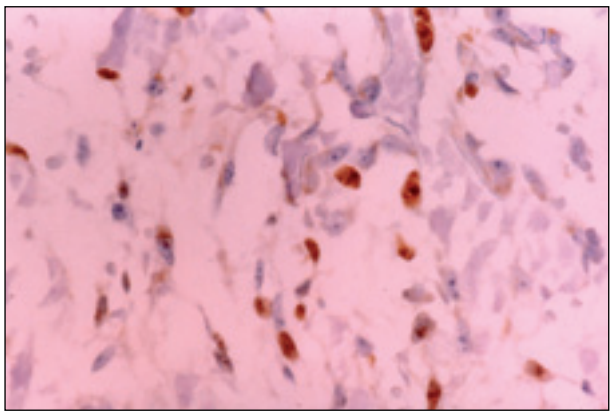

Fig. 4. Intracartilagineous ossification: CD30 positive cells of the perichondrium at the site of primary ossification center. Immunostain x 200

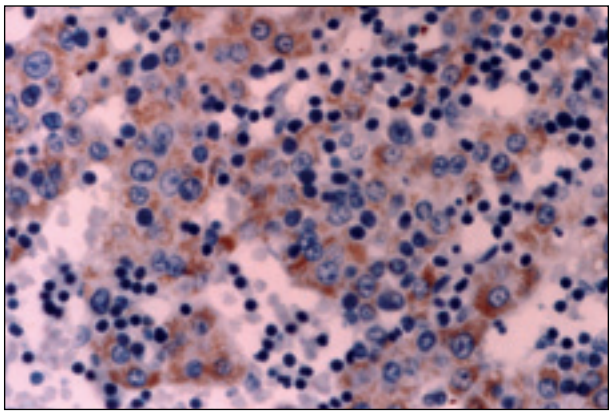

Fig. 2. Liver: CD30 is expressed in liver parenchymal cells, but not in hematopoietic cells. Immunostain x 200

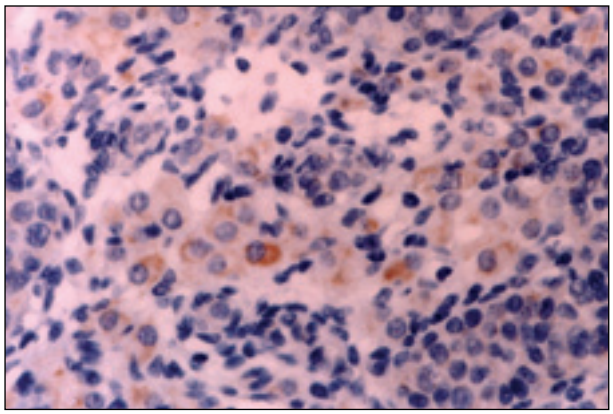

Fig. 5. Ovary: Cuboidal or elongated CD30 positive cells in the cortex of the ovary. Immunostain x 200

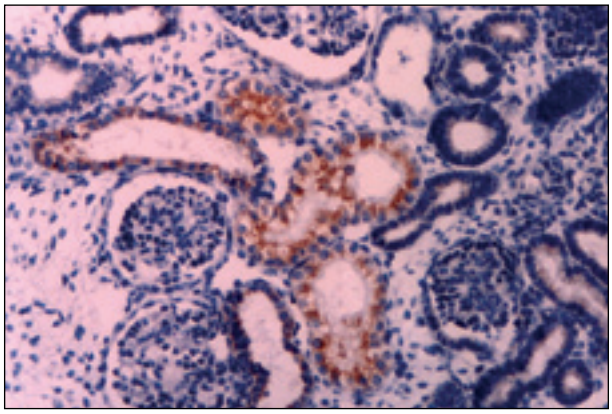

Fig. 3. Kidney: CD30 expression in the epithelial cells of tubules and ducts in the fetal kidney (cortex). Immunostain x 200

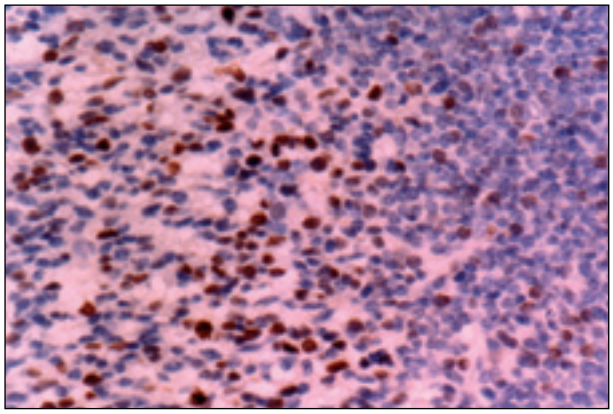

Fig. 6. Neural tissue: In the cerebral cortex, CD30 was expressed predominantly by neurons near the surface. Immunostain x 200

CD30 polygonal mesenchymal cells were observed within a loosely organized connective tissue stroma. In the reproductive system, in both male and female embryos at the 10th week of gestation, staining for CD30 antigen was limited to a population of cuboidal or elongated cells distributed within the cortex of the ovary (Fig. 5),

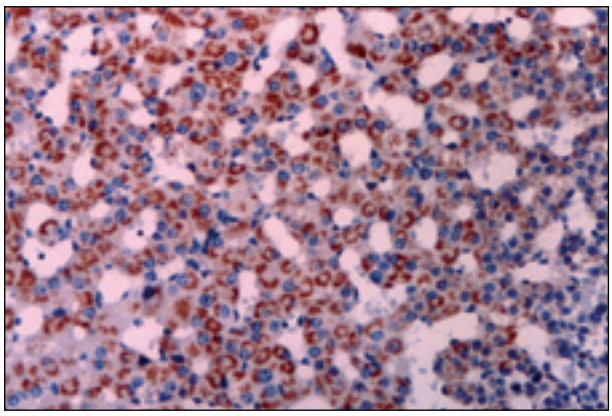

Fig. 7. Adrenal gland: Strong staining for CD30 in medullary cells. Immunostain x 200

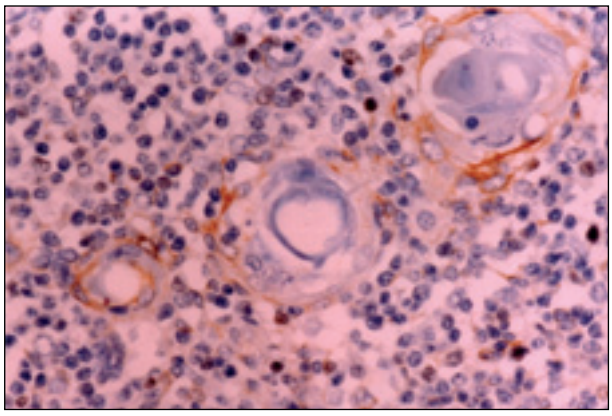

Fig. 8. Thymus: CD30 immunoreactive Hassal's corpuscles in the thymic medulla. Immunostain x 400

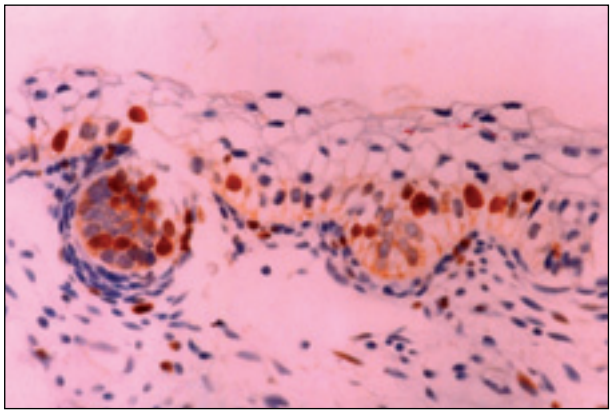

Fig. 9. Skin: The skin of a 10th week fetus showing restriction of CD30 expression in the basal layer. Immunostain x 200

and medulla of the testis. In the nervous system (cerebral cortex and cerebellum), CD30 was detected in cerebral cortical neurons (Fig. 6), and Purkinje cells. In the endocrine system (adrenal gland), medullary cells showed a strong cytoplasmic positivity for CD30 antigen, sparing the cortex (Fig. 7). In the haematolymphoid system (thymus), both thymic epithelial cells and Hassal's corpuscles in the medulla showed high CD30 expression (Fig. 8). In the thymic cortex, staining was limited to a population of flattened, elongated cells located under the connective tissue capsule of the organ. This is consistent with the observation that human thymus is not completely differentiated before week 13th of gestation, when the cortico-medullary junctions and the first Hassal corpuscles become visible. In the skin of the 10-week fetuses, in which the epidermis is comprised of only 2 cell layers, CD30 was expressed in the cells of the basal layer only (Fig. 9). In 16-week fetuses, staining was predominantly observed in the developing skin adnexa (basal cells of epidermal buds), and in occasional basal keratinocytes.

Other tissues

The immunohistochemical control for the detection of CD30 antigen in the developing respiratory and cardiovascular system was negative.

DISCUSSION

During embryogenesis, CD30 could be found in derivatives of all three germ layers; however, this expression was not ubiquitous. Ectodermal derivatives that contained CD30 included cells of the central nervous system, medulla of the adrenal gland, and epidermis (7). Mesodermal tissues expressing CD30 included kidney, primary ossification centers, and gonads. Intestine, liver, pancreas, and thymus - the endoderm-derived tissues, also expressed CD30 antigen (7, 8). There was an early CD30 expression in several fetal tissues derived from all three germ layers (8th to 10th gestational week) with the exception of the skin and thymus in which the antigen was demonstrated later on. The late expression in the skin and thymus could be attributed to the late full development of these organs: 10th week onwards for the skin and 13th week onwards for the thymus.

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 (9). Shared expression of

CD30 antigen does not necessarily relate Hodgkin and R-S cells to activated lymphocytes (9, 10). 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 tissues suggests that previous theories as to the nature of the CD30 antigen must be re-examined. The Hodgkin and Reed-Sternberg cells are indeed lymphocytes as they harbor rearranged immunoglobulin in more than 90% of cases and T cell receptors (11). Although the expression of CD30 antigen may indicate a relationship between these cell types, it is likely to be less straightforward than previously supposed. Identification of the normal physiologic role of CD30 antigen is thus made even more imperative if these relationships are to be understood.

Another point is that outside the lymphatic system, CD30 antigen expression in the epithelial cells of developing fetal tissues can mediate signals for cell proliferation and differentiation in a region where other cell types grow throughout life, for example in the case of intestinal cryptal we refer to stem, goblet, Paneth and enteroendocrine cells (8, 13–15).

CD30 also appears to be expressed in a selected group of terminally differentiated cells, which are responsive to hormonal stimulation (fetal skin keratinocytes) (16–19). This variation of expression suggests a possible role for hormones, preferably progesterone, in the regulation of CD30 expression (19). This would be a novel mechanism of CD30 induction other than neoplastic transformation and viral infection of lymphocytes. In our previous investigation concerning the developing intestinal crypts, the demonstration of the large Reed-Sternberg like cells in the developing crypts within a lymphoplasmacytic infiltrate, in the same way that similar Reed-Sternberg 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 Reed- Sternberg cells (8).

The possibility that CD30 antigen is an oncofetal antigen is supported by our positive findings in fetal tissues. We have been able to investigate several tissues from a number of fetuses from 8th gestational week onwards. Pallesen and Hamilton-Dutoit [14] 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 is the first demonstration of CD30 in epithelial cells in fetal tissue.

Our findings of Ber-H2 staining of fetal tissues and organs could well be added to the list of non- lymphoid tissues and cells, both normal and neoplastic, expressing CD30 antigen. Moreover, when considered together with reported staining seen in placenta (4, 5), 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.

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XXXI. Angiologické Dny 2006

Kongresové centrum Praha, 23.–25. 2. 2006

Hlavní téma: Cévní onemocnění a jejich patogeneze, diagnostika, prevence a terapie

Vybraná témata a vyzvané přednášky: Ateroskleróza jako systémové onemocnění - Neinvazivní a invazivní diagnostické postupy u cévních pacientů - Rizikové faktory aterosklerózy - Prevence kardiovaskulárních komplikací - Protilipidová léčba u pacientů s onemocněním periferních tepen - Doporučení pro léčbu pacientů s intermitentními klaudikacemi - Kritická končetinová ischémie - Diabetická noha - Současný stav léčby aneuryzma aorty - Cerebrovaskulární onemocnění - Pokroky v intervenční angiologii - Plicní hypertenze - Nové léky u žilní tromboembolické nemoci - Prevence žilní trombózy u interních a chirurgických pacientů - Trombotické postižení povrchových žil - Primární varixy a posttrombotický syndrom - Chronická žilní insuficience a bércové vředy - Moderní přístupy u pacientů s lymfedémem - Kompresivní léčba - Změny mikrocirkulace za klinických stavů - Duplexní sonografie tepen a žil - Raynaudův syndrom - Práce angiologické sestry

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