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CD30 Antigen in Embryonal Carcinoma and Embryogenesis and Release of the Soluble Molecule

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The expression, serological detection, and possible functional role of the CD30 antigen in Hodgkin's disease and anaplastic large cell lymphoma is well documented. In embryonal carcinoma (EC), the expression of this cytokine receptor has been demonstrated only by immunohistology. Because the CD30 monoclonal antibody Ki-1 was found to cross-react with an unrelated molecule, we examined by in situ hybridization testicular germ cell neoplasms for the presence of CD30-specific transcripts. CD30 mRNA was detectable in the tumor cells of 9 of 9 cases of EC or mixed germ cell tumors with an EC component but in no other nonlymphoid tumors. Thus, the CD30 transcript expression pattern proved to be identical to the immunostaining pattern seen with the CD30-specific monoclonal antibody Ber-H2. By Northern blot analysis, CD30 transcripts could be demonstrated in the EC cell line Tera-2. Employing a highly sensitive second generation sandwich enzyme-linked immunosorbent assay, we could detect the soluble CD30 molecule in 8 of 8 sera from patients with a diagnosis of EC but not in 8 of 10 sera from patients with other testicular germ cell tumors. In fetal tissue, no CD30-expressing germ cells or epithelial cells could be observed. Thus, the cellularly expressed CD30 molecule appears to represent a real tumor marker for testicular neoplasms of EC type. Moreover, the serum levels of soluble CD30 antigen seem to be a promising parameter for monitoring patients with EC. (Am J Pathol 1995, 146: 463–471)

The value of the CD30 antigen as a diagnostic marker for Hodgkin's disease and anaplastic large cell lymphoma is well documented.[1–3] In normal cells, this transmembrane glycoprotein can be induced on B and T lymphocytes by mitogen stimulation or viral transformation.[4–6] The cDNA cloning revealed that the CD30 protein is a cytokine receptor of the tumor necrosis factor receptor superfamily,[7,8] the ligand of which belongs to the tumor necrosis factor family.[9,10] Recent *in vitro* data indicate that the CD30 receptor-ligand complex can mediate signals for cell proliferation, apoptosis, and cytotoxicity in lymphoid cells.[9–11] However, the function of this new cytokine receptor in Hodgkin's disease and other CD30-positive diseases is still not clear. Moreover, in analogy to other members of the tumor necrosis factor receptor family,[12–14] a soluble form of the CD30 molecule (sCD30) is released, probably due to enzymatic cleavage. This soluble molecule can be detected in extracellular fluids.[15] Increased serum levels have been reported to occur in patients with CD30-positive lymphomas[15–21] and during the active phase of infectious mononucleosis.[19]

Initial evidence for a strong and frequent expression of the CD30 protein in embryonal carcinoma (EC) came from Pallesen and Hamilton-Dutoit.[22] They described immunoreactivity of CD30 monoclonal antibodies (MAbs) in 12 of 14 cases of primary or meta-

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static EC or mixed germ cell tumors with EC elements. Preliminary data on the CD30 antigen expression in normal embryonal cells gave negative results.[22] More recently, reactivity of testicular EC with the antibody Ber-H2 has also been described.[23] However, whether the observed reactivity of CD30 MAbs with the cells of ECs really represented the expression of the CD30 molecule or alternatively was due to a cross-reactivity of the antibodies used was not clarified. From the investigations of Rohde et al,[24] we know that the Ki-1 MAb, apart from recognizing the CD30 antigen, reacts with a biochemically different molecule, the nucleic kinase named Ki-1/57. To help clarify the situation, we investigated by means of *in situ* hybridization (ISH) germ cell tumors for the presence of CD30-specific transcripts. We compared the ISH results with those obtained by immunostaining with the CD30 Ber-H2 MAb. In addition, we immunostained embryonal tissues of various gestation stages. Moreover, to contribute to a potential diagnostic and to a therapeutic value of our findings, we determined the serum concentration of sCD30 in normal individuals and in patients suffering from testicular germ cell tumors.

Materials and Methods

Reagents

All media, supplements, and enzymes were purchased from GIBCO BRL (Karlsruhe, Germany) and Boehringer Mannheim (Mannheim, Germany) unless otherwise indicated. Radiochemicals were from DuPont-New England Nuclear (Bad Homburg, Germany).

Tissue Sampling

Orchiectomy specimens of 27 patients with testicular germ cell tumors (for diagnosis see Table 1) were routinely processed by formol fixation and paraffin embedding in the Institute of Pathology, Klinikum Benjamin Franklin. Small parts of tumor tissue were snap frozen in liquid nitrogen. The age of the patients ranged from 16 to 53 years (median, 27 years). Classification of germ cell tumors was done according to the World Health Organization International Histological Classification of Tumours.[25] As positive control for ISH served four cases of Hodgkin's disease (lymph nodes, two cases each of mixed cellularity and nodular sclerosis subtype), as negative control one case of each of the following: undifferentiated spindle cell sarcoma of the uterus, serous adenocarcinoma of the ovary, pre-B lymphoblastic lymphoma of the testis, centroblastic lymphoma (lymph node), immunoblastic lymphoma (vulva) and large cell carcinoma of the lung. The normal embryonal tissues were collected at the Institute of Pediatric Pathology, Universitätsklinikum Rudolf Virchow. For ISH, cryostat sections were air dried on a hot plate at 55 C for a few minutes and fixed in 4% paraformaldehyde/phosphate-buffered saline for 20 minutes. Slides were rinsed with phosphate-buffered saline and dehydrated in graded ethanols.

Cell Cultures

Cells were maintained in RPMI 1640 containing 10% (v/v) fetal calf serum supplemented with 4 mmol/L glutamine, 500 U/ml penicillin, and 100 µg/ml streptomycin. The cell line Tera-2, which is derived from metastasis of the lung of a patient with EC, was obtained from the American Type Culture Collection (Rockville, MD), and L428 and L591 were kindly supplied by Prof. Dr. Volker Diehl, Cologne, Germany.[26,27]

RNA Probes

For the preparation of single-stranded RNA probes, a CD30 cDNA probe (nucleotides (nt) 1156 to 3385 of the published sequence)[7] was subcloned into the vector pGEM1 (Promega Biotec, Madison, WI). After linearization of the plasmid with appropriate restriction enzymes, anti-sense and sense (control) RNA probes were generated by run-off transcription by using SP6 or T7 RNA polymerase with incorporation of ^{35}S-labeled nucleotides as described.[28] For ISH, probes with a specific activity of approximately 1.2×10^9 cpm/µg were used.

ISH and Autoradiography

ISH was performed as described.[28] In brief, cryostat sections or dewaxed and rehydrated paraffin sections were exposed to 0.2 N HCl and 125 µg/ml Pronase (Boehringer), followed by acetylation with 0.1 mol/L triethanolamine (pH 8.0) and 0.25% (v/v) acetic anhydride and dehydration through graded ethanols. Slides were hybridized to 2×10^5 to 4×10^5 cpm of labeled probes overnight at 50 C. Slides were subsequently washed in high stringency conditions, treated with RNAse A, coated with Ilford G5 radiographic emulsion (Ilford, Mobberley, UK), exposed at 4 C for 2 to 5 weeks, developed in Kodak D19 developer (Kodak, Hemel Hampstead, UK), and counterstained in hematoxylin and eosin. The incubation of

sections with *Micrococcus* nuclease (Boehringer) before ISH resulted in the extinction of the specific autoradiographic signal, establishing that RNA sequences were the targets of the hybridization procedure.[29] The hybridization of the *Ava*-I/*Hin*dIII fragment of the TNF-α probe (American Type Culture Collection) to macrophages and lymphoid cells served as positive control for the presence of RNA. Additional control experiments consisted of hybridizations with the sense probe that showed only weak background staining (Figure 1).

Northern Blot Hybridization Analysis

Poly(A)$^+$ RNA isolation, size fractioning, and transfer was performed as described earlier.[7] Hybridizations were carried out with [^{32}P]dCTP-labeled CD30 cDNA

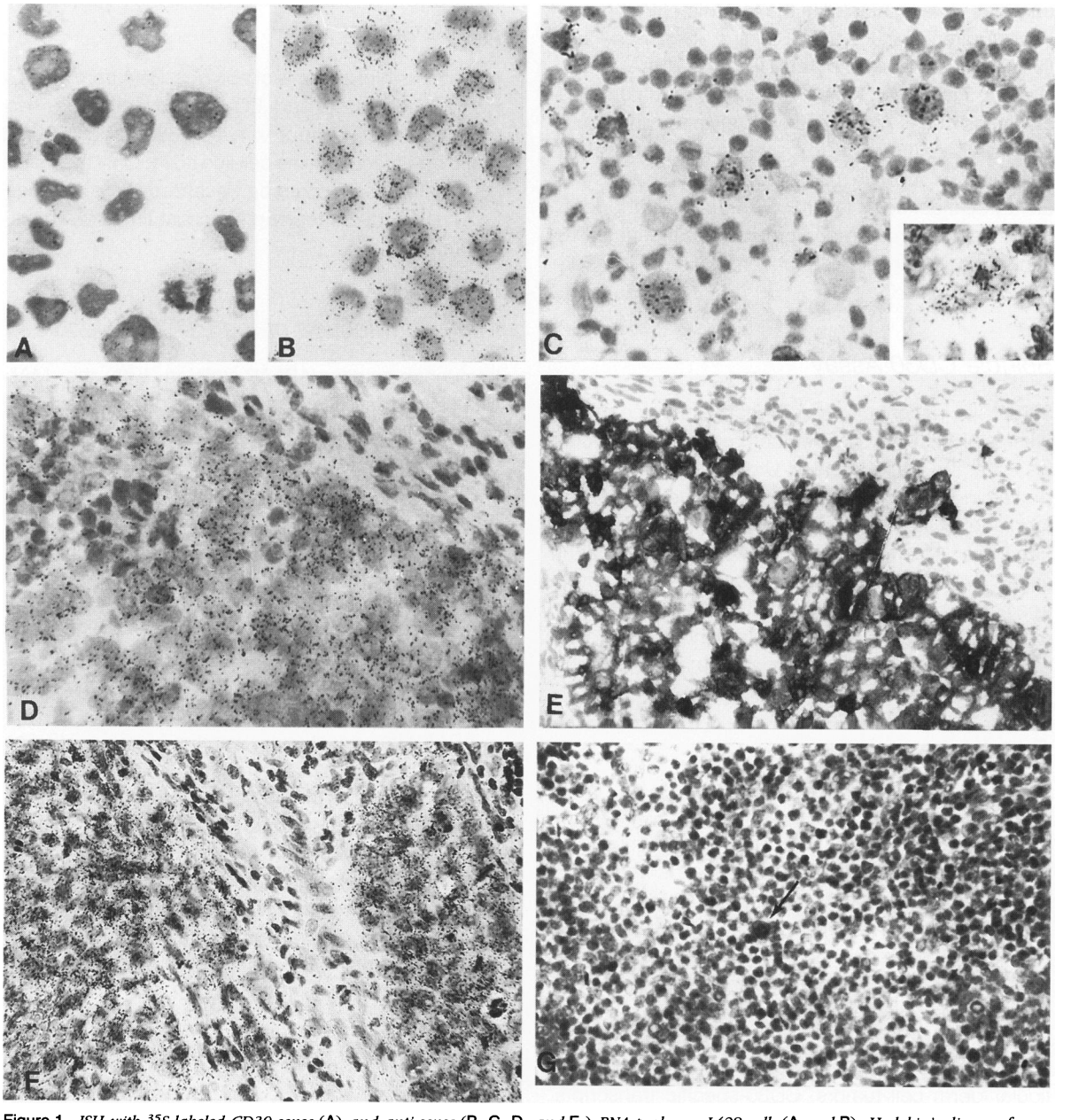

Figure 1. *ISH with ^{35}S-labeled CD30 sense* (A) *and anti-sense* (B, C, D, *and* F) *RNA probe on L428 cells* (A *and* B); *Hodgkin's disease, frozen tissue* (C); *Reed-Sternberg cell, formalin-fixed tissue* (C, insert); *EC, frozen tissue* (D); *and EC, formalin-fixed tissue* (F) *and APAAP immunostaining with Ber-H2 MAb on adjacent section of EC, frozen tissue* (E) *and on fetal thymus, week 14 of gestation, formalin-fixed tissue* (G, *arrow marks single CD30-positive lymphoid blast in the medulla). Magnifications, ×300* (A *and* C), *×420* (B), *×240* (D), *×160* (E *and* F), *×120* (G).

inserts (nt 1 to 1913, and nt 1156 to 3385 of the published sequence).[7]

Immunostaining

CD30 MAbs used for this investigation were Ber-H2,[3] Ki-1,[1] HRS-4[30] (a generous gift of Prof. M. Pfreundschuh, Homburg, Germany), and S9.13–1[31] (kindly donated by Dr. H. Lemke, Kiel, Germany). The MAbs Ber-H2 and Ki-1 and the AP3 antibody of the alkaline phosphatase anti-alkaline phosphatase (APAAP) complex were produced in the Institute of Pathology, Klinikum Benjamin Franklin. Staining of formalin-fixed tissue was performed by applying the APAAP technique.[32] Frozen sections from germ cell tumors and cytospins of cell line Tera-2 were stained with immunoperoxidase (DAKO, Glostrup Denmark),[4] because endogenous alkaline phosphatase could not sufficiently be blocked by levamisole. Paraffin-embedded tissue sections required microwave treatment for 15 minutes in 0.1 mol/L citrate buffer, pH 6, for MAb Ber-H2.

Soluble CD30 Assay

Sera from patients with malignant germ cell neoplasms of the testis obtained before surgery were collected at the Department of Urology at the Klinikum Benjamin Franklin. Soluble antigen levels were determined by means of a new highly sensitive second generation sandwich enzyme-linked immunosorbent assay (DAKO) that is based on two MAbs directed against different epitopes of the sCD30. Normal sera, obtained from eight healthy male donors, served as a negative control (values between 0 and 20 U/ml).

Results

In Situ *Detection of the CD30 mRNA*

Demonstration of the CD30 mRNA in the tumor cells of four patients with Hodgkin's disease, as well as the Hodgkin's disease-derived cell line L428 (Figure 1), served as a positive control for the establishment of the CD30 *in situ* detection. Furthermore, normal tonsils and lymph nodes were examined. CD30-specific signals were detectable only in the few CD30-positive lymphoid blasts at the rim of germinal centers.

Applying ISH to sections of nine patients with testicular germ cell tumors, CD30-specific transcripts were detected in all EC cells, whereas stromal cells remained unlabeled (Table 1A, Figure 1). In mixed germ cell tumors, only the EC component was positive. On other tumor components, such as teratoma, seminoma, choriocarcinoma, carcinoma *in situ* (TIN), and yolk sac tumor, only background signals were observed. No specific signal was demonstrated in any CD30-negative tumor entity tested, as judged by immunohistological staining on serial sections. All data were in agreement with parallel immunostaining results on serial sections.

Detection of the CD30 mRNA by Northern Blot Analysis

In Northern blot analysis of poly(A)$^+$ RNA from human Hodgkin's disease and EC-derived cell lines, two mRNA species of approximately 3800 and 2600 nt could be detected in the cell line L591. Only the major 3800-nt transcript could be visualized in the cell lines L428 and Tera-2 after long exposure times (4 weeks, Figure 2).

Immunodetection of the CD30 Molecule

Immunoperoxidase/APAAP-staining with the MAb Ber-H2 was carried out on 27 cases of testicular germ cell tumors as well as cytospins of the cell line Tera-2 (Table 1). Only the tumor cells of EC (3 cases), the EC component of mixed germ cell tumors (14 cases), and the EC cell line were CD30 positive, whereas the tumor cells of other neoplasias of the testis were negative (10 cases). Occasionally, a few reactive CD30-positive lymphoid blasts were detectable in seminomas. Additionally, the MAbs Ki-1, HRS-4, and S9.13–1 were applied to adjacent sections of 4 cases. Here, the immunostaining with different CD30 MAbs gave similar results.

APAAP immunostaining with the MAb Ber-H2 was carried out on microwave-treated sections of paraffin-embedded tissue of fetal liver, lung, kidney, gastrointestinal tract, adrenal gland, lymph nodes, thymus, spleen, gonads, and brain from week 6 of gestation onwards. Only large lymphoid blasts in lymph nodes and the medulla of the thymus from week 14 of gestation onwards were immunostained, whereas all other cells remained negative (Figure 1).

Detection of sCD30 Antigen by ELISA

As shown in Table 1B, increased serum levels of sCD30 (mean +/– SD) were found in 8 of 8 patients with the diagnosis of EC or mixed germ cell tumor with

Table 1. *Detection of CD30*
A: Immuno- and in situ *labeling of CD30*

Diagnosis		Immunostaining of tumor cells	*In situ* labeling of tumor cells
Hodgkin's disease (n = 4)		+	+
Embryonal carcinoma			
Case 1: EC, CC, YS	(F)	+*	+*
Case 2: EC, CS	(F)	+*	+*
Case 3: EC, TE	(F)	+*	+*
Case 4: EC, CS	(F)	+*	+*
Case 5: EC, SE	(F)	+*	+*
Case 6: EC	(F)	+	+
Case 7: EC, TE, YS, SE	(P)	+*	+*
Case 8: EC	(P)	+	+
Case 9: EC, CC	(P)	nd	+*
Other neoplasia			
Teratoma (n = 1)	(F)	–	–
Uterine sarcoma (n = 1)	(F)	–	–
Large cell lung carcinoma (n = 1)	(F)	–	–
Ovarian adenocarcinoma (n = 1)	(F)	–	–
Pre-B NHL (n = 1)	(F)	–	–
CB (n = 1)	(F)	–	–

B: Immunodetection of cellular and soluble CD30 antigen

Diagnosis		Immunostaining of tumor cells	Serum levels of sCD30 (U/ml)
Healthy donor (n = 8)		nd	0–20
Embryonal carcinoma			
Case 10: EC, CC	(P)	+*	36
Case 11: EC, TE, SE	(P)	+*	36
Case 12: EC, TE, YS, SE	(P)	+*	39
Case 13: EC, SE, YS	(P)	+*	50
Case 14: EC	(P)	+	55
Case 15: EC, SE, YS	(P)	+*	96
Case 16: EC, TE	(P)	+*	160
Case 17: EC, TE	(P)	+*	270
Other germ cell tumors			
Case 18: SE	(P)	–	0
Case 19: SE	(P)	–	0
Case 20: SE	(P)	–	0
Case 21: SE	(P)	–	4
Case 22: SE, TE	(P)	–	7
Case 23: TE	(P)	–	10
Case 24: SE, CS	(P)	–	13
Case 25: SE	(P)	–	14
Case 26: TE	(P)	–	25
Case 27: TE	(P)	–	46

P, paraffin sections; F, frozen sections; CC, choriocarcinoma; YS, yolk sac tumor; TE, teratoma; CS, carcinoma *in situ* (TIN); SE, seminoma; CB, centroblastic lymphoma; NHL, non-Hodgkin's lymphoma; *, only labeling of EC component; nd, not determined.

an EC component (93 +/– 83 U/ml; range, 36 to 270 U/ml), whereas it was absent from 8 of 10 cases with other malignant tumors of the testis (6 +/– 6 U/ml; range, 0 to 14 U/ml). However, serum concentrations from 2 patients with teratoma without an EC component (cases 26 and 27 from Table 1B) were also slightly increased (25 U/ml and 46 U/ml).

Discussion

Testicular germ cell tumors with EC differentiation have been described to react with the CD30 antibodies Ki-1[22] and Ber-H2.[22,23] As the antibody Ki-1 reacts with a biochemically different molecule, Ki-1/57,[24] it is not clear whether this staining represents a true expression of the CD30 molecule. In the current study, we could demonstrate specific CD30 transcripts in all germ cell tumors that consisted exclusively of EC cells or in mixed germ cell tumors with an EC component. The results were confirmed by Northern blot analysis of the EC cell line Tera-2. Here, we could detect the larger transcript of the differentially spliced CD30 mRNA,[7] although there was only a small amount of this RNA. The smaller minor CD30 transcription product might have been beneath the detection limit of the Northern blot. Thus, the data on the immunoreactivity of CD30 MAbs with EC, presented in this paper and those previously described,[22,23] are

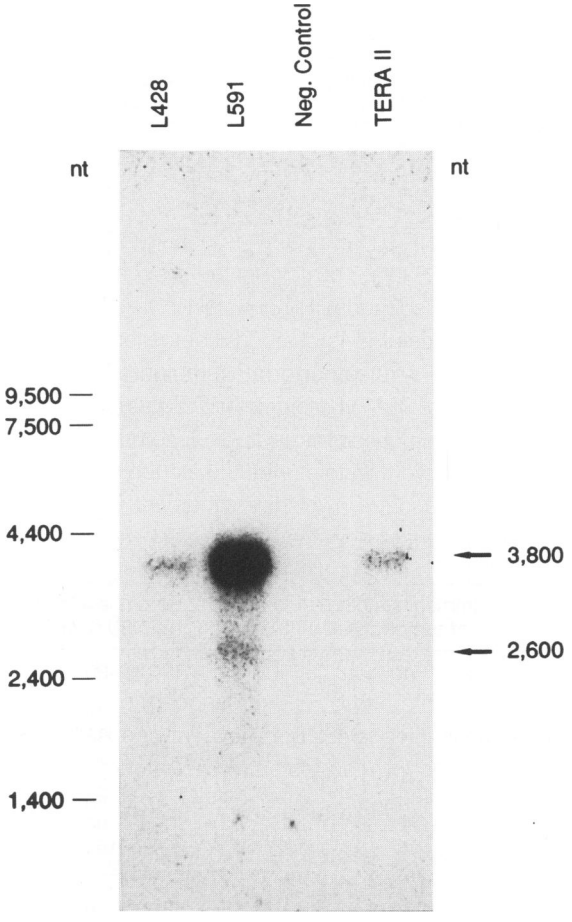

Figure 2. *Northern blot analysis from poly(A)⁺ RNA of L428, L591, and Tera-2 cell lines with ^{32}P-labeled CD30 inserts. Autoradiographic exposure time was 4 weeks.*

definitely not due to cross-reactivity but due to CD30 mRNA transcription and translation.

Reactivity with some CD30 antibodies has been described to occur in other nonlymphoid malignancies[3,33,34] and in nonlymphoid cells.[35,36] Studies at the RNA level similar to the one presented here may help clarify whether these reactivities represent true CD30 expression or cross-reactivities with unrelated antigens. We would, however, like to point out that most of these results were either nonreproducible or inconsistent, with the possible exception of the recent detection of CD30 in decidua.[36] Thus, Schwarting and co-workers (1989)[3] reported a weak Ber-H2 reactivity on paraffin sections of pancreatic carcinomas, salivary gland carcinomas, malignant melanomas, and breast carcinomas. In the same paper it is mentioned that no confirmation of these results was produced when the staining was repeated with the Ber-H2 and Ki-1 MAbs on frozen material.[3] Mechtersheimer and Möller[33] described the expression of the CD30 antigen in several mesenchymal tumors. These results were obtained with the MAb Ki-1 on frozen sections. It was not possible for the mentioned authors to reproduce these staining results on the same tissues in our laboratory (G. Mechtersheimer and H. Stein, unpublished results). Finally, Andreesen and colleagues[35] reported Ki-1 reactivity on human blood monocytes that had been cultured on hydrophobic Teflon foils for more than 8 days. In our hands, the same staining was obtained with an unrelated, subgroup-matched, murine IgG3 antibody (F. Eitelbach, unpublished data). No reactivity of these stimulated monocytes was revealed when stained with another murine CD30 MAb (Ber-H2) belonging to the IgG1 subclass (F. Eitelbach, unpublished data). Moreover, monocytes and macrophages are consistently CD30 negative in frozen and paraffin-embedded tissue sections.

After confirming that the MAb Ber-H2 detects only the CD30 molecule and no other unrelated antigen in EC, we extended our immunohistological analysis to the expression of CD30 during normal embryogenesis. For this purpose, normal fetal tissues from the 6th week of gestation onwards were systematically immunohistologically stained with the Ber-H2 MAb. We found a population of CD30-positive large lymphoid blasts in lymphoid tissue from the 14th week onwards with a morphology, frequency, and distribution similar to that observed in normal adult tissue. The difference of our results to earlier preliminary investigations by Pallesen and Hamilton-Dutoit[22] might be due to our very sensitive immunostaining procedure based on an improved antigen retrieval by microwave treatment of paraffin-embedded tissue. However, we did not find any expression of the CD30 molecule in the gonads or in any epithelial organ. It should be noted that we cannot rule out CD30 expression in earlier stages of the embryonal development as no specimens covering the period before the 6th week were available. With this limitation in mind, the expression of the CD30 molecule in EC cells appears not to reflect an embryonic differentiation stage. Instead, it is more likely that CD30 expression is related to malignant transformation, especially in view of the fact that the telomeric part of the short arm of chromosome 1, where the CD30 gene is located,[37] is often altered in malignancies.[38] In support of this possibility is the finding that the Tera-2 cell line exhibits a deletion of 1p among other chromosomal abnormalities.[39] For the substantiation of this possibility, cytogenetic investigations on freshly obtained EC material are necessary.

Based on the fact that the CD30 molecule is a new cytokine receptor that can mediate signals for cell proliferation or apoptosis,[10] it is intriguing to further

speculate on the role of this protein in the pathogenesis of EC. In conjunction with other events, the expression of the CD30 molecule might eventually lead to the neoplastic process. Reports on the expression of other growth factors and their receptors, eg, fibroblast growth factor, transforming growth factor, and platelet-derived growth factor in EC cell lines[40] make it conceivable that autocrine or paracrine growth factor regulation plays a role in the pathogenesis of EC. In this respect, it will be interesting to analyze the expression of the CD30 ligand[9,10] in testicular germ cell tumors.

Next, we investigated the occurrence of the soluble form of the CD30 antigen[15] in sera from patients suffering from germ cell tumors of the testis. From investigations of other CD30-positive neoplasms, including Hodgkin's disease,[19–21] anaplastic large cell lymphoma,[19] angioimmunoblastic lymphoma,[17] and adult T cell lymphoma/leukemia,[16,19] it is known that increased serum levels of sCD30 correlate with the tumor burden. Thus, sCD30 serum analysis can be used for the monitoring of the therapeutic effects.[41] In our study, we found low (20 to 50 U/ml) and moderate (50 to 100 U/ml) serum levels in three cases each and high values (100 to 270 U/ml) in sera from two patients with the diagnosis of EC. No sCD30 could be detected in sera from healthy controls and the majority of patients with pure seminomas and other germ cell neoplasms of the testis. However, two cases of teratoma displayed slightly elevated levels of sCD30, although the tumor cells were negative for CD30 as judged by immunohistochemical staining. The reason for this elevation is not clear. We assume that the increased serum concentrations are due to an obscure EC component in the tumor that was not met in the tissue sections analyzed. Another reason for the elevated sCD30 serum level could be an increase of CD30-positive lymphoid blasts caused by a viral infection, such as Epstein-Barr virus[19] or a hepatitis virus.[42]

Analysis of sCD30 levels in patients with Hodgkin's disease has recently yielded interesting results.[21] In contrast to earlier investigations,[18,20] the authors of this study observed elevated serum levels at the time of presentation in 87% of the patients studied by using a new more sensitive enzyme-linked immunosorbent assay test kit. Moreover, the risk of treatment failure was significantly higher in patients with serum concentrations greater than 100 U/ml. A similarly designed follow-up study for patients suffering from EC has been planned that should clarify whether the determinations of serum sCD30 levels can help, as in the case of Hodgkin's disease, towards a clinically relevant characterization and monitoring of malignant testicular diseases.

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References

1. Schwab U, Stein H, Gerdes J, Lemke H, Kirchner HH, Schaadt M, Diehl V: Production of a monoclonal antibody specific for Hodgkin and Sternberg-Reed cells of Hodgkin's disease and a subset of normal lymphoid cells. Nature 1982, 299:65–67
2. Stein H, Mason DY, Gerdes J, O'Connor N, Wainscoat J, Pallesen G, Gatter K, Falini B, Delsol G, Schwarting R, Lennert K: The expression of Hodgkin's disease associated antigen Ki-1 in reactive and neoplastic lymphoid tissue: evidence that Reed-Sternberg cells and histiocytic malignancies are derived from activated lymphoid cells. Blood 1985, 66:848–858
3. Schwarting R, Gerdes J, Dürkop H, Falini B, Pileri S, Stein H: Ber-H2: a new anti-Ki-1 (CD30) monoclonal antibody directed at a formol-resistant epitope. Blood 1989, 74:1678–1689
4. Stein H, Gerdes J, Schwab U, Lemke H, Mason DY, Ziegler A, Schienle W, Diehl V: Identification of Hodgkin and Sternberg-Reed cells as a unique cell type derived from a newly-detected small cell population. Int J Cancer 1982, 30:445–449
5. Froese P, Lemke H, Gerdes J, Havensteen B, Schwarting R, Hansen H, Stein H: Biochemical characterization and biosynthesis of the Ki-1 antigen in Hodgkin-derived and virus-transformed human B and T lymphoid cell lines. J Immunol 1987, 139:2081–2087
6. Andreesen R, Osterholz J, Lohr GW, Bross KJ: A Hodgkin cell-specific antigen is expressed on a subset of auto- and alloactivated T (helper) lymphoblasts. Blood 1984, 63:1299–1302
7. Dürkop H, Latza U, Hummel M, Eitelbach F, Seed B, Stein H: Molecular cloning and expression of a new member of the nerve growth factor receptor family that is characteristic for Hodgkin's disease. Cell 1992, 68: 421–427
8. Mallet S, Barclay AN: A new superfamily of cell surface proteins related to the nerve growth factor receptor. Immunol Today 1991, 12:220–223
9. Beutler B, van Huffel C: Unraveling function in the TNF ligand and receptor families. Science 1994, 264:667–668
10. Smith CA, Gruss HJ, Davis T, Anderson D, Farrah T, Baker E, Sutherland GR, Brannan CI, Copeland NG, Jenkins N, Grabstein KH, Gliniak B, MacAlister IB, Fanslow W, Alderson M, Falk B, Gimpel S, Gills S, Din WS, Goodwin RG, Armitage RJ: CD30, a marker for

Hodgkin's lymphoma, is a receptor whose ligand defines an emerging family of cytokines with homology to TNF. Cell 1993, 73:1349–1360
11. Bowen MA, Olsen KJ, Lirong Cheng, Avila D, Podack ER: Functional effects of CD30 on a large granular lymphoma cell line, YT. J Immunol 1993, 11:5896–5906
12. Nophar Y, Kemper O, Brakebusch C, Engelmann H, Zwang R, Aderka D, Holtmann H, Wallach D: Soluble forms of tumor necrosis factor receptors (TNF-Rs): the cDNA for the type I TNF-R, cloned using amino acid sequence data of its soluble form, encodes both the cell surface and a soluble form of the receptor. EMBO J 1990, 9:3269–3278
13. Loenen WAM, De Vries E, Gravenstein LA, Hintzen RQ, Van Lier RAW, Borst J: The CD27 membrane receptor, a lymphocyte-specific member of the nerve growth factor receptor family, gives rise to a soluble form by protein processing that does not involve receptor endocytosis. Eur J Immunol 1992, 22:447–455
14. Kohno T, Brewier M, Baker S, Schwartz PE, King MW, Hale KK, Squires CH, Thompson RC, Vannice JL: A second tumor necrosis factor receptor gene product can shed a naturally occurring tumor necrosis factor inhibitor. Proc Natl Acad Sci USA 1990, 87:8331–8335
15. Josimovic-Alasevic O, Dürkop H, Schwarting R, Backé E, Stein H, Diamantstein T: Ki-1 (CD30) antigen is released by Ki-1-positive tumor cells *in vitro* and *in vivo*. I. Partial characterization of soluble Ki-1 antigen and detection of the antigen in cell culture supernatants and in serum by an enzyme-linked immunosorbent assay. Eur J Immunol 1989, 19:157–162
16. Dallenbach F, Josimovic-Alasevic O, Dürkop H, Schwarting R, Diamantstein T, Matsuoka M, Takatsuki K, Stein H: Soluble CD30 antigen in the sera of patients with adult T-cell lymphoma/leukemia (ATL): a marker for disease activity. Leukocyte Typing IV. Edited by W Knapp, B Dörken, W Gilks, EP Rieber, RE Schmidt, H Stein, REGK van dem Borne. Oxford, Oxford University Press, 1989, pp 426–428
17. Pizzolo G, Stein H, Josimovic-Alasevic O, Vinante R, Zanotti R, Chilosi M, Feller AC, Diamantstein T: Increased serum levels of soluble IL-2 receptor, CD30 and CD8 molecules, and γ-interferon in angioimmunoblastic lymphadenopathy: possible pathogenetic role of immunoactivation mechanisms. Br J Haematol 1990, 75:485–488
18. Pizzolo G, Vinante F, Chilosi M, Dallenbach F, Josimovic-Alasevic O, Diamantstein T: Serum levels of soluble CD30 molecule (Ki-1 antigen) in Hodgkin's disease: relationship with disease activity and clinical stage. Br J Haematol 1990, 75:282–284
19. Pfreundschuh M, Pohl C, Berenbeck C, Schroeder J, Jung W, Schmits R, Tschiersch A, Diehl V, Gause A: Detection of a soluble form of the CD30 antigen in sera of patients with lymphoma, adult T-cell leukemia and infectious mononucleosis. Int J Cancer 1990, 45: 869–874
20. Gause A, Pohl C, Tschiersch A, DaCosta L, Diehl V, Hasenclever D, Pfreundschuh M: Clinical significance of soluble CD30 antigen in the sera of patients with untreated Hodgkin's disease. Blood 1991, 77:1983–1988
21. Nadali G, Vinante F, Ambrosetti A, Todeschini G, Veneri D, Meneghini V, Ricetti MM, Benedetti F, Vassanelli A, Perona G, Chilosi M, Menestrina F, Fiacchini M, Stein H, Pizzolo G: Serum levels of soluble CD30 are elevated in the majority of untreated patients with Hodgkin's disease and correlate with clinical features and prognosis. J Clin Oncol 1994, 12:793–797
22. Pallesen G, Hamilton-Dutoit SJ: Ki-1 (CD30) antigen is regularly expressed by tumor cells of embryonal carcinoma. Am J Pathol 1988, 133:446–450
23. Ferreiro JA: Ber-H2 expression in testicular germ cell tumors. Hum Pathol 1994, 25:522–524
24. Rohde D, Hansen H, Hafner M, Lange H, Mielke V, Hansmann M-L, Lemke H: Cellular localizations and processing of two molecular forms of the Hodgkin-associated Ki-1 (CD30) antigen: the protein kinase Ki-1/57 occurs in the nucleus. Am J Pathol 1992, 140: 473–482
25. Mostofi FK, Sobin LH: Histological typing of testis tumours. World Health Organization, Geneva, 1977
26. Diehl V, Kirchner HH, Burrichter H, Stein H, Fonatsch C, Gerdes J, Schaadt M, Heit W, Uchanska-Ziegler B, Ziegler A, Heintz F, Sueno K: Characteristics of Hodgkin's disease derived cell lines. Cancer Treat Rep 1982, 66:615–632
27. Diehl V, Kirchner HH, Fonatsch C, Stein H, Gerdes J, Boie C: Hodgkin's disease: establishment of four *in vitro* cell lines. J Cancer Res Clin Oncol 1981, 101: 111–124
28. Milani S, Herbst H, Schuppan D, Hahn EG, Stein H: *In situ* hybridization for collagen types I, III and IV mRNA in normal and fibrotic rat liver: evidence for predominant expression in non-parenchymal liver cells. Hepatology 1989, 10:84–92
29. Williamson DJ: Specificity of riboprobes for intracellular RNA hybridization. Histochemistry 1988, 26:84–92
30. Schwarting R, Stein H: Cluster report: CD30. Leukocyte typing IV. Edited by W Knapp, B Dörken, W Gilks, EP Rieber, RE Schmidt, H Stein, AEGK van dem Borne. Oxford, Oxford University Press, 1989, pp 419–422
31. Horn-Lohrens O, Lange H, Hafnert W, Sterry W, Parwaresch R, Lemke H: Clustering of idiotypes on the CD30 (Ki-1) antigen. Second International Symposium on Hodgkin's disease. Edited by V Diehl. Köln, 1991
32. Cordell J, Falini B, Erber ON, Gosh AK, Abdulazi Z, Macdonald S, Polford K, Stein H, Mason DY: Immunoenzymatic labelling of monoclonal antibodies using immune complexes of alkaline phosphatase and monoclonal anti-alkaline phosphatase (APAAP complex). J Histochem Cytochem 1984, 32:219–229

33. Mechtersheimer G, Möller P: Expression of Ki-1 antigen (CD30) in mesenchymal tumors. Cancer 1990, 66:1732–1737
34. Sohail D, Simpson RHW: Ber-H2 staining of lipoblasts. Histopathology 1991, 18:191
35. Andreesen R, Brugger W, Löhr GW, Bross KJ: Human macrophages can express the Hodgkin's cell-associated antigen Ki-1 (CD30). Am J Pathol 1989, 134:187–192
36. Ito K, Watanabe T, Hovie R, Shiota M, Kawamura S, Mori S: High expression of the CD30 molecule in human decidual cells. Am J Pathol 1994, 145:276–280
37. Fonatsch C, Latza U, Dürkop H, Rieder H, Stein H: Assignment of the human CD30 (Ki-1) gene to 1p36. Genomics 1992, 14:825–826
38. Fonatsch C: Cytogenetics of malignant lymphomas. Blut 1988, 57:101–109
39. Lai-che Wang, Vass W, Changlie G, Chang KSS: Amplification and enhanced expression of the c-Ki-*ras2* protooncogene in human embryonal carcinomas. Cancer Res 1987, 47:4192–4198
40. Miller WH, Dimitrovsky E: Growth factors in human germ cell tumors. Recent Res Cancer Res 1991, 123: 183–189
41. Gause A, Jung W, Schmits R, Tschiersch A, Scholz R, Pohl C, Diehl V, Pfreundschuh M: Soluble CD8, CD25 and CD30 antigens as prognostic markers in patients with untreated Hodgkin's lymphoma. Ann Oncol 1992, 3:49–51
42. Pizzolo G, Vinante F, Morosato L, Nadali G, Chilosi M, Gandini G, Sinicco A, Raiteri R, Semenzato G, Stein H, Perona G: High serum level of the soluble form of CD30 molecule in the early phase of HIV-1 infection as an independent predictor of disease progression. AIDS 1994, 8:741–745