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Review article

Towards the phenotyping of soft tissue tumours by cell surface molecules*

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Summary. This study is aimed at the characterization of soft tissue tumours (STT) by means of cell surface molecules. To achieve this, normal mesenchymal tissues were extensively examined for expression of leucocyte differentiation (CD) antigens and HLA molecules. The panel of antigens finally examined in STT comprised CD10, CD13, CD24, CD34, CD36, CD56, CD57, HLA-A,B,C, β_2-microglobulin, HLA-DR, -DP, and -DQ and the HLA-D-associated invariant chain (Ii). STT were determined by conventional histomorphological and immunohistochemical criteria. The immunohistological analysis was based on serial frozen sections, one of which was used to demonstrate CD53 antigen. This very broadly distributed leuco/histiocyte-restricted antigen allowed for the distinction between the background of interstitial "stromal" cells and the neoplastic population. In some STT, the expression pattern of the cell surface molecules corresponded to that in their non-neoplastic counterparts. The majority of STT, however, showed considerable changes in the cell surface immunophenotype compared to their cells of origin. These alterations consisted mainly in an aberrant induction/neoexpression and, to a much lesser extent, in an aberrant down-regulation/loss of cell surface antigens. Nevertheless, some immunophenotype configurations are described which, for the time being, can be considered to be useful supplements in the differential diagnosis of this complex class of tumours. The data also indicate considerable changes in cell surface antigen expression occurring in the course of neoplastic transformation of mesenchymal cells. Detailed analysis of alterations in the functional repertoire of neoplastic mesenchymal cells might provide new insights into the biology of STT, possibly leading to new concepts for therapeutic intervention.

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Key words: Soft tissue tumours – Phenotyping – Cell surface molecules

Introduction

Soft tissue tumours (STT) represent a heterogeneous and complex group of neoplasms which are usually classified according to a variable degree of morphological similarity with the presumed normal tissue of origin. The variety of histopathological manifestations has led to a wide range of tumour types and subtypes listed in the currently used classifications (Hajdu 1979; Enzinger and Weiss 1988). Although different histogenetically, various STT may have similar morphology. Thus, the histological classification of STT, especially of undifferentiated, highly malignant sarcomas, remains a challenge even to the experienced pathologist.

Surgery has been the first and only valuable treatment for STT for decades. The outcome of this treatment was influenced by localization and local extent and, to a lesser degree, by the histologically determined malignant potential rather than by the detailed subclassification of the neoplasms. During the past few years, induction chemotherapy has emerged as a well-established tool for sarcomas of childhood and adolescents, using specific detailed treatment protocols for different tumour types (e.g. Sawaguchi et al. 1990). Additionally, neo-adjuvant and/or postoperative chemotherapy, frequently combined with radiotherapy, is now often applied to sarcomas in the adult (reviewed by Verweij et al. 1989; Bramwell 1989). For these complex therapeutic modalities an accurate subclassification of STT is necessary. Detailed knowledge of the biochemical characteristics and, in the majority of cases, of the molecular structure of surface antigens of haematopoietic cells and of cytokine receptors produced by or acting on immune cells has opened a new dimension in the understanding of leukaemias and lymphomas (Lennert and Feller 1990). At regularly held workshops on human leucocyte differentiation anti-

gens, cluster of differentiation (CD) designations are assigned to monoclonal antibodies that produce identical cellular reaction patterns and identify the same molecular structure. It is now known that a considerable number of CD antigens are identical to cell-adhesion molecules, enzymes, cytokine or even growth factor receptors (reviewed by Knapp et al. 1989). This knowledge has led to the design of new immunotherapeutic treatment modalities which are extensively applied to haematopoietic malignancies. The well-defined CD antigens on normal and malignant lymphoid cells are important targets in antibody-directed therapy, using mono- and bispecific monoclonal antibodies and conjugates for the treatment of leukaemia. Likewise, cytokines have been successfully used in both lymphoma and leukaemia (reviewed by Borden and Sondel 1990; Ferrini et al. 1990). Soluble CD antigens have been detected in sera of patients with lymphoma/leukaemia and have been revealed to be an indicator of disease activity (Dallenbach et al. 1989). Meanwhile, immunotherapy is also increasingly applied to carcinomas and malignant melanomas (for review see Borden and Sondel 1990; Ferrini et al. 1990). Monoclonal antibodies directed against tumour-associated antigens have been shown to help in the diagnostic imaging and treatment of neuroblastomas (e.g. Kemshead et al. 1986).

The evaluation of the possible application of immunotherapy to STT treatment requires a detailed knowledge of the cell surface immunophenotype of these neoplasms which, at the same time, might serve as a first step towards an analysis of the functional morphology of STT. The characterization of the cell surface immunophenotype of STT might also help in the differential diagnosis of this intricate tumour group.

Given the above considerations, this study is an attempt towards phenotyping a comprehensive series of STT by means of cell surface molecules including a variety of well-characterized CD antigens as well as major histocompatibility (MHC) class I and class II determinants.

Materials and methods

Tissue. Representative frozen tissue samples of a comprehensive series of normal tissues containing all kinds of mesenchymal cells, of 25 benign and 103 malignant STT, were collected over a period of 3.5 years. Tissue sections were quick-frozen in liquid nitrogen and stored at −70° C. Frozen sections of about 1 cm^2 and a thickness of 4–6 µm were air-dried, acetone-fixed at room temperature for 10 min, then immunostained immediately or stored at −20° C for a short period.

Classification of the STT. Diagnosis of each STT was established on routine paraffin sections (H&E, periodic acid-Schiff, Masson-Goldner and Gomori silver stains) according to standard histopathological criteria (Enzinger and Weiss 1988) in conjunction with immunohistochemical analysis using a broad spectrum of mono- and polyclonal antibodies directed against well-established structural antigens of mesenchymal cells (for review see Enzinger and Weiss 1988; Wick and Swanson 1988).

Reagents. The monoclonal antibodies (mAbs) used in this study are listed in Table 1. mAb HEA125 (IgG1 isotype) and HD77 [IgM(G1) isotype] were raised in the laboratory of P. Möller (in collaboration with F. Momburg/G. Moldenhauer and B. Dörken/G. Moldenhauer, respectively). mAb My10 (IgG1 isotype). Leu-19 (IgG1 isotype) and Leu-7 (IgM isotype) were obtained from Becton-Dickinson (Mountain View, Calif.). mAb J5 (IgG2a isotype) and My7 (IgG1 isotype) were purchased from Coulter Immunology (Hialeah, Fl)., mAb OKB2 (IgG1 isotype) and OKM5 (IgG1 isotype) were provided by Ortho Diagnostics (Raritan, N.J.). The mAb against MHC class I and class II antigens, β_2-microglobulin (β_2m) and HLA-D-associated invariant chain (Ii) were generous gifts from the producing laboratories (Table 1); mAb B7/21 (IgG1 isotype) was purchased from Becton-Dickinson and mAb Tü22 (IgG2a isotype) was supplied by Biotest (Dreieich, FRG). mAb HD39 (IgG1 isotype), mAb W6/32.HK (IgG2a isotype), a non-binding variant of the anti-HLA-A,B,C mAb W6/32, and mAb B2 (IgM isotype), which were used as isotype-matched control antibodies, were kindly provided by B. Dörken/G. Moldenhauer (Heidelberg, FRG), A. Ziegler (Marburg, FRG) and L.N. Nadler (Boston, Mass.), respectively. A polyclonal biotinylated sheep antibody to mouse Ig (reactive with all mouse isotypes) and a streptavidin-biotinylated peroxidase complex were provided by Amersham (High Wycombe, UK). 3-Amino-9-ethylcarbazole (AEC) and NN-dimethylformamide (DMF) were obtained from Sigma Chemicals (St. Louis, Mo.).

Staining procedure. For staining, following rehydration with phosphate buffered saline solution (PBS; pH 7.5), the frozen sections were incubated for 1 h with culture supernatant (ISCR3, BBM.1) or purified mAb (1 mg/ml) at appropriate dilutions [HEA125(1:100), HD77(1:100), My10(1:20), OKM5(1:20), J5(1:50), My7(1:50), Leu-7(1:20), Leu-19(1:20), OKB2(1:20), W6/32(1:100), B7/21(1:20), Tü22(1:20), VIC-Y1(1:100)]. The sections were then incubated with biotinylated anti-mouse Ig (1:50) and streptavidin-biotinylated peroxidase complex (1:100) for 30 min, respectively. All incubation steps were carried out in a humid chamber at room temperature and followed by double rinsing in PBS. The peroxidase reaction product was reddish-brown in colour when AEC and hydrogen peroxide were used as substrate: 4 mg AEC was dissolved in 500 µl DMF. Subsequently, 9.5 ml sodium acetate-buffer (0.05 M; pH 5.0) and, just before use, 5 µl hydrogen peroxide (30%) was added. Incubation time was 15 min. The sections were then rinsed in distilled water, counterstained with Harris' haematoxylin and mounted in glycerol gelatin.

Controls. Negative controls in each case were performed without primary antibody; no staining was observed except for scattered granulocytes. This staining was caused by endogenous peroxidase, which was not blocked for the benefit of optimal antigenicity. The positive results were confirmed by controls using the isotype-matched mAb HD39(1:100), W6/32.HK(1:100) and B2(1:100), which yielded negative results in all cases, ruling out non-specific antibody binding. Additionally, intrinsic positive controls for the immunoreactivity of MHC class-I and class-II antigens and for Ii were scattered dendritic interstitial cells (DIC) and lymphoid cells. Endothelial cells, tissue histiocytes, scattered fibrocytes and, at least in some cases, entrapped nerve fibres, served as positive intrinsic controls for the immunoreactivity of CD34, CD36, CD53, CD10, CD13, CD24, CD56 and CD57 antigens, respectively.

Evaluation. Evaluation of the staining intensity of the tumour cells corresponding to the MHC antigenic density in and on them was carried out against the background of DIC that were ubiquitously present and always strongly stained. Thus, three grades of intensity were scored: (1) strong staining, +; (2) weak staining, (+); and (3) no staining, −. Positive and negative tumour cells present in various amounts were considered +/−. The expression of CD antigens in tumour cells was also evaluated in a semi-quantitative fashion: +, strong and/or weak positivity of the entire tumour cell population; +/−, positive and negative tumour cells in various amounts; −, negativity of the entire tumour cell population.

Table 1. List of antigens detected in this study

Antigen	Clone	Reference	Mol.wt.	Molecular structure/comment (for review see Knapp et al. 1989; Krensky et al. 1990)
Egp34	HEA125	Momburg et al. 1987	34 kDa	Epithelium-specific cell surface glycoprotein
CD53	HD77	Hadam et al. 1989	32–40 kDa	Pan leuco-histiocyte single chain glycoprotein
CD34	My10	Civin et al. 1984	115 kDa	Single chain transmembrane glycoprotein showing no significant similarity with any described cell surface molecule. Suggested to be involved in cell-matrix interactions.
CD36	OKM5	Talle et al. 1983	90 kDa	Single chain membrane glycoprotein corresponding to platelet GPIV (GPIIIb). Identified as primary receptor for collagen.
CD10	J5	Ritz et al. 1981	100 kDa	Type II integral membrane glycoprotein; 750 amino acid sequence with a single 24 amino acid hydrophobic segment. Identical with neutral endopeptidase/enkephalinase, which inactivates several peptide hormones including glucagon, enkephalins, substance P, neurotensin, oxytocin and bradykinin.
CD13	My7	Griffin et al. 1981	150 kDa	Integral single chain glycoprotein; 967 amino acid sequence with a single 24 amino acid hydrophobic segment. Identical with aminopeptidase N, an important enzyme of brush border membranes of the small intestine, renal proximal tubules, and placenta, also found on synaptic membranes of the CNS.
CD56	Leu-19	Lanier et al. 1986	220/140 kDa	Cell surface glycoprotein corresponding to the NKH-1 molecule. Identical with the 140 kDa isoform of neural-cell adhesion molecule (N-CAM)
CD57	Leu-7	Abo and Balch 1981	110 kDa	Cell surface glycoprotein corresponding to the HNK-1 molecule. Recognizes the myelin-associated glycoprotein (MAG) and detects a sulphated carbohydrate epitope expressed on a portion of N-CAM.
CD24	OKB2	Mittler et al. 1983	36/40 kDa	Single chain glycoprotein with phosphoinositol-linked functional structure.
β_2m	BBM.1	Brodsky et al. 1979	11.5 kDa	Non-polymorphic β-chain of major histocompatibility complex (MHC) class I molecules
HLA-DR	ISCR3	Watanabe et al. 1983	28/34 kDa	α/β heterodimer of MHC class II antigens.
HLA-DP	B7/21	Royston et al. 1981	28/34 kDa	α/β heterodimer of MHC class II antigens.
HLA-DQ	Tü22	Ziegler et al. 1982	28/34 kDa	α/β heterodimer of MHC class II antigens.
Ii	VIC-Y1	Quaranta et al. 1984	28/34 kDa	Intracellulary, MHC class II antigens are non-covalently associated with the non-polymorphic invariant chain (Ii). VIC-Y1 recognizes the intracytoplasmic domain of Ii, the surface domain of which is recognized by CD74 mAb.

Results and comments

The patterns of expression of all antigens in normal tissue are shown in Table 2. The detailed distribution pattern of CD10 and CD13, of CD56 and CD24 as well as of HLA-A,B,C and β_2m molecules in STT is given in Tables 3–5, respectively. HLA-A,B,C and β_2m determinants displayed a comparable distribution pattern and are thus dealt with jointly.

Egp34 antigen

Non-neoplastic mesenchymal cells. All non-neoplastic mesenchymal cells were Egp34-negative throughout.

Soft tissue tumours. Except for a monophasic fibrous synovial sarcoma which expressed Egp34 in a minor tumour cell population, the STT were consistently Egp34-negative.

Comment. Egp34 is a broadly distributed epithelium-specific cell surface glycoprotein which is considered to be absent from all extra-epithelial cell types (Momburg et al. 1987). In accordance with these data, all non-neoplastic mesenchymal cells and, excepting a single case of monophasic fibrous synovial sarcoma, all STT studied lacked any detectable Egp34 molecules. This finding suggests that Egp34 is a very useful tool to differentiate between STT and anaplastic carcinomas. Its diagnostic value is stressed by the fact that a variety of STT exhibit a co-expression of cytokeratins (e.g. Norton et al. 1987). Thus, Egp34 is by far superior to cytokeratins in the differential diagnosis between STT and carcinomas.

CD53 antigen

Non-neoplastic mesenchymal cells. The CD53 molecule was expressed by all lymphocytes and tissue histiocytes/macrophages. Based on their more or less elongated cy-

Table 2. Expression of cell surface molecules in non-neoplastic mesenchymal cells

Phenotype	CD53	CD34	CD36	CD10	CD13	CD57	CD56	CD24	HLA-A, B, C/β_2m	HLA-D/Ii
Smooth muscle cells										
– vessels	–	–	–	–	–	–	–	–	–	–
– intestine	–	–	–	–	–	–	(+)/–	–	–	–
– uterus, prostate	–	–	–	–	–	–	(+)/–	–	(+)	–
Striated muscle cells										
– fetal skeletal muscle	–	–	+	–	–	–	+	–	–	–
– adult skeletal muscle	–	–	(+)/–	–	–	–	–/(+)	–	–	–
– regenerative skeletal muscle	–	–	+/–	–	–	–	+/(+)	–>(+)	–	–
– cardiac muscle	–	–	(+)	–	–	–	+	–	–	–
Fibrocytes	–	+/–	–	+/–	+/–	–	–	–	+	–
Histiocytes	+	–	+	–	+/–	–	–	–	+	+
Chondrocytes	–	–	–	–	–	–	–	–	–	–
Osteoblasts	–	–	–	–	–	–	–	–	+	–
Osteoclasts	+	–	–	–	+	–	–	–	+	–
Peripheral nerves										
– thin nerve fibres	–	–	–	+/(+)	–	–	+	+	+	+/–[a]
– thick nerve fibres	–	–	–	+/(+)	–	+	–	–	–	–
Ganglion cells	–	–	–	–	–	–	+/–	–	–	–
Satellite cells	–	–	–	–	–	–	+	+	+	–
Adrenal medulla										
– fetal chromaffin cells	–	–	–	–	–	+/–	+	+	–	–
– adult chromaffin cells	–	–	–	–	–	+/–	+	+	+	–
Adipocytes	–	–	+	–	–	–	–	–	–	–
Endothelial cells										
– vascular endothelial cells	–	+	+	–	–	–	–	–	+	+/–[b]
– lymphatic endothelial cells	–	–	–	–	–	–	–	–	–	–

[a] HLA-DR and -DP positivity in some cases
[b] HLA-DR positivity in some cases

toplasmic processes the latter were designated DIC (Fig. 1). Similarly, osteoclasts and osteoclast-like giant cells were CD53-positive, whereas all other mesenchymal cells lacked any detectable CD53 molecules.

Soft tissue tumours. In the overwhelming majority of STT studied, the neoplastic population was CD53-negative throughout. Only 4 of 12 malignant fibrous histiocytomas and 2 of 4 osteosarcomas displayed a CD53-positive minor tumour cell subpopulation (Fig. 2), which included osteoclast-like giant cells in 2 malignant fibrous histiocytomas. Moreover, some scattered osteoclast-like giant cells observed in 1 leiomyosarcoma were CD53-positive. In contrast to the mononuclear tumour cell population, which expressed desmin, these CD53-positive osteoclast-like giant cells lacked any detectable desmin (unpublished data).

Comment. The CD53 antigen detected by mAb such as HD77 has recently been defined as a pan leuco/histiocyte antigen (Hadam 1989). In situ, it is expressed by all lymphocytes, tissue histiocytes/macrophages and by all bone marrow cells including osteoclasts. There are no data available on the expression of CD53 in non-neoplastic or neoplastic extrahaematopoietic mesenchymal cells (Hadam 1989). In accordance with these data, CD53 allowed the reliable and distinct visualization of the lympho-histiocytic stromal infiltrate in both non-neoplastic mesenchymal tissues and in STT. Since other stromal cells including fibrocytes/-blasts and (collapsed) endothelial cells as well as the overwhelming majority of neoplastic cells were CD53-negative throughout, serial frozen sections permitted their clear discrimination from CD53-positive lympho-histiocytic stromal infiltrates. What is more, CD53 revealed that STT, in contrast to other solid tumours, generally appeared devoid of significant inflammatory infiltrates. CD53 expression in some malignant fibrous histiocytomas and osteosarcomas does not disturb the concept of the complete absence of CD53 in extrahaematopoietic mesenchymal cells, since osteosarcomas and, at least according to some authors (e.g. Shirasuna et al. 1985), also malignant fibrous histiocytomas, are considered parts of the monocyte/macrophage system. In addition to its value in the visualization of the stromal infiltrate in STT, CD53 is a useful tool in the differential diagnosis between CD53-negative STT and haematopoietic malignancies, which are generally CD53-positive (P. Möller, personal communication).

CD34 antigen

Non-neoplastic mesenchymal cells. CD34 antigen was consistently expressed by endothelial cells of capillaries

Table 3. Expression of CD10 and CD13 antigens in neoplastic cells of soft tissue tumours (STT)

Phenotype	n	CD10(J5)			CD13(My7)			CD10/CD13 co-expression
		+	+/−	−	+	+/−	−	
Leiomyoma	4	3	1	−	−	2	2	2
Leiomyo-blastoma	1	−	−	1	1	−	−	−
Leiomyo-sarcoma	16	9	4	3	3	5	8	7
Rhabdomyo-sarcoma	8	−	3	5	−	−	8	−
Fibroma	2	−	−	2	−	−	2	−
Aggressive fibromatosis	3	1	2	−	1	2	−	3
Fibro-sarcoma	4	−	2	2	−	2	2	1
Synovial sarcoma	5	1	2	2	1	1	3	1
Malignant fibrous histiocytoma	12	10	2	−	2	7	3	9
Chondro-sarcoma	3	−	−	3	−	−	3	−
Osteo-sarcoma	4	1	3	−	1	3	−	4
Neurilemoma	3	−	1	2	−	−	3	−
Granular cell tumour	1	−	−	1	−	−	1	−
Malignant schwannoma	14	1	3	10	1	4	9	3
Peripheral neuroepithelioma	5	−	−	5	−	−	5	−
Ewings's sarcoma	1	1	−	−	−	1	−	1
Ganglio-neuroma	4	−	−	4	−	−	4	−
Ganglio-neuroblastoma	5	−	−	5	−	−	5	−
Neuro-blastoma	4	−	−	4	−	−	4	−
Lipoma	2	−	−	2	−	−	2	−
Lipsarcoma	11	3	4	4	2	3	6	2
Lymphangioma	1	−	−	1	−	−	1	−
Haemangioma	4	−	−	4	−	−	4	−
Haemangio-pericytoma	6	−	−	6	−	−	6	−
Haemangio-sarcoma	1	−	−	1	−	−	1	−
Epithelioid sarcoma	1	−	−	1	1	−	−	−
Alveolar soft part sarcoma	1	−	−	1	−	−	1	−
Clear cell sarcoma	2	−	−	2	−	−	2	−

Table 4. Expression of CD56 and CD24 antigens in neoplastic cells of STT

Phenotype	n	CD56(Leu-19)			CD24(OKB2)			CD56/CD24 co-expression
		+	+/−	−	+	+/−	−	
Leiomyoma	4	2	1	1	1	−	3	1
Leiomyo-blastoma	1	1	−	−	−	−	1	−
Leiomyo-sarcoma	16	9	1	6	3	−	13	1
Rhabdomyo-sarcoma	8	6	2	−	−	−	8	−
Fibroma	2	1	1	−	−	−	2	−
Aggressive fibromatosis	3	−	3	−	−	−	3	−
Fibro-sarcoma	4	3	−	1	−	−	4	−
Synovial sarcoma	5	3	1	1	1	−	4	1
Malignant fibrous histiocytoma	12	1	4	7	−	1	11	1
Chondro-sarcoma	3	−	2	1	−	1	2	1
Osteo-sarcoma	4	1	3	−	−	4	−	4
Neurilemoma	3	2	1	−	−	−	3	−
Granular cell tumour	1	−	1	−	−	−	1	−
Malignant schwannoma	14	4	6	4	2	3	9	3
Peripheral neuroepithelioma	5	−	1	4	−	1	4	1
Ewing's sarcoma	1	−	−	1	−	−	1	−
Ganglio-neuroma	4	4	−	−	−	−	4	−
Ganglio-neuroblastoma	5	5	−	−	5	−	−	5
Neuroblastoma	4	4	−	−	4	−	−	4
Lipoma	2	−	−	2	−	−	2	−
Liposarcoma	11	2	3	6	2	4	5	3
Lymphangioma	1	−	−	1	−	−	1	−
Haemangioma	4	−	−	4	−	−	4	−
Haemangio-pericytoma	6	−	2	4	−	−	6	−
Haemangio-sarcoma	1	−	−	1	−	−	1	−
Epithelioid sarcoma	1	−	1	−	−	−	1	−
Alveolar soft part sarcoma	1	−	−	1	−	−	1	−
Clear cell sarcoma	2	−	−	2	−	−	2	−

and small blood vessels. In addition, endothelial cells of medium-sized blood vessels were optionally CD34-positive, whereas those of large blood vessels and of lymph vessels, regardless of size, were CD34-negative throughout. CD34 molecules, in some non-neoplastic tissues, were also found in scattered fibrocytes and reticular fibres, for example, in the uterus and in the skin surrounding cutaneous appendages.

Soft tissue tumours. In the vast majority of STT studied, CD34 was strongly and consistently expressed by endothelial cells of the capillary network of the tumours and of the vascular proliferates of 4/4 haemangiomas (Fig. 3). Small and some medium-sized blood vessels showed also CD34-positive endothelial cells in one lymphangioma, whereas those of proliferating lymph vessels lacked any detectable CD34 molecules. Further-

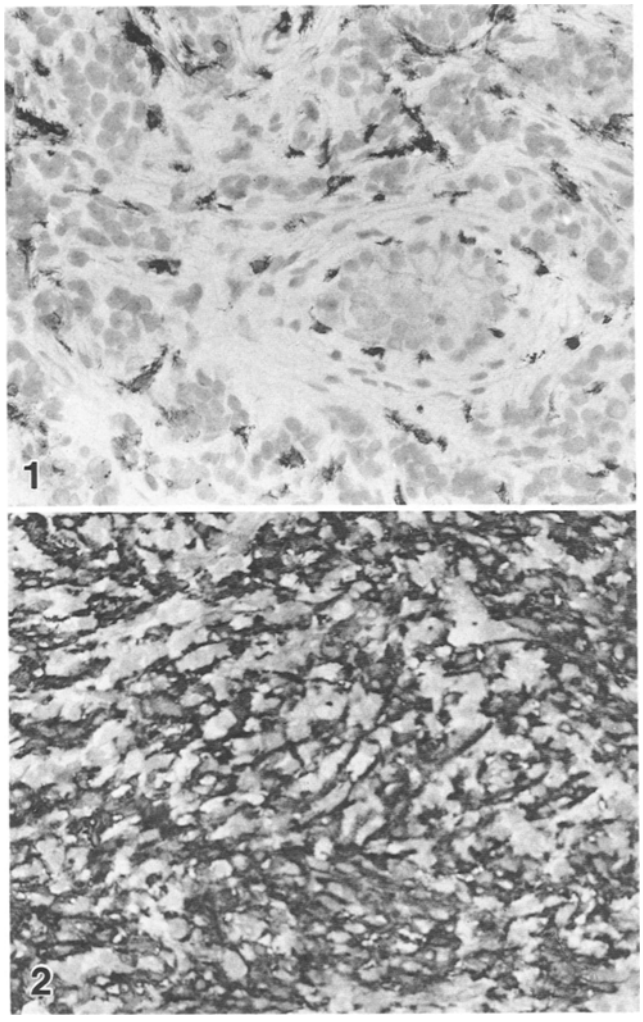


Fig. 1. Testicular rhabdomyosarcoma. MAb HD77 reveals strongly CD53-positive dendritic interstitial cells (DIC). Small, round, undifferentiated tumour cells and seminiferous tubules are CD53-negative. ×225

Fig. 2. Storiform-pleomorphic malignant fibrous histiocytoma of the upper extremity. HLA-DP recognized by mAb B7/21 is expressed on a major tumour cell population. ×180

more, 1 haemangiosarcoma was CD34-positive throughout (Fig. 4), as was the haemangioendotheliomatous component present in 1 of 6 haemangiopericytomas. Besides their expression in vascular structures and their neoplastic counterparts, CD34 molecules were found in all tumour cells of 2 of 16 leiomyosarcomas and 4 of 14 malignant schwannomas as well as in at least a tumour cell subpopulation of 1 further malignant schwannoma and 1 of 5 monophasic fibrous synovial sarcomas. In some STT, CD34 molecules were also expressed by perivascular fibrocytes, and scattered fibrocytes of adjacent pseudo-capsules and of fibrosing areas expressed CD34 molecules.

Comment. CD34 antigen is a powerful human haematopoietic progenitor cell antigen exhibiting stage- rather than lineage-specific characteristics (Civin et al. 1984). Outside the haematopoietic system, expression of CD34 has been demonstrated in endothelial cells, especially of capillaries, and in an haemangiosarcoma while absent in various other solid tumours (Beschorner et al. 1985; Watt et al. 1987; Fina et al. 1990; Schlingemann et al. 1990). Moreover, some CD34 expression was found in stromal matrix or reticular fibres and was also assumed on extended processes of fibroblasts (Fina et al. 1990). In this study, CD34 proved to be a reliable marker for capillary endothelial cells in both non-neoplastic and neoplastic tissues while lacking in endothelial cells of lymph vessels. In addition to the vascular stromal compartment, at least some stromal fibrocytes were optionally CD34-positive in both non-neoplastic and neoplastic tissues, adding further evidence for CD34 expression in fibrous tissues. Neoplastic fibrous cells, however, were consistently CD34-negative except for the fibro-cellular stroma of 5 fibroadenomas of the breast (unpublished data). It is noteworthy that CD34 expression in an haemangiosarcoma and in the haemangioendotheliomatous component of an haemangiopericytoma corresponds to the findings in non-neoplastic vascular endothelial cells, thus confirming CD34 expression in vascular neoplasms. Since normal smooth muscle cells, peripheral nerves and adipocytes were CD34-negative throughout the neo-expression of CD34 in some STT derived from these cell types cannot be explained at present. Obviously, CD34 is helpful in elucidating the vascular component of the tumour stroma and the neo-vascularization in STT and also represents a useful supplement in the diagnosis of haemangiosarcomas. Nevertheless, the optional expression of these molecules in a few other STT should not be disregarded.

CD36 antigen

Non-neoplastic mesenchymal cells. Like the CD34 molecule, the CD36 antigen was consistently expressed in endothelial cells of capillaries and small blood vessels while being completely absent in the endothelium of lymph vessels. Strong expression of CD36 molecules was also determined in adipocytes, in cardiac muscle cells as well as in fetal and in some regenerative skeletal muscle cells. Adult skeletal muscle fibres showed only weak and inconsistent CD36 expression. Some scattered tissue histiocytes/macrophages were also CD36 positive.

Soft tissue tumours. As was the case with CD34, CD36 molecules were expressed in endothelial cells of the capillary network and of proliferating small and medium-sized blood vessels in STT while lacking in the endothelium of proliferating lymph vessels in 1/1 lymphangiomas. One haemangiosarcoma and 2 of 6 haemangiopericytomas, including 1 case with focal haemangioendotheliomatous differentiation, were also consistently CD36-positive. CD36 molecules were likewise detected in all tumour cells of 2 of 2 lipomas and 4 of 11 liposarcomas, including all ($n=3$) of myxoid and 1 of pleomorphic subtype (Fig. 5). Similarly, in 2 of 8 rhabdomyosarcomas, 1 of embryonal, the other of pleomorphic subtype, some rounded or elongated rhabdomyoblasts were

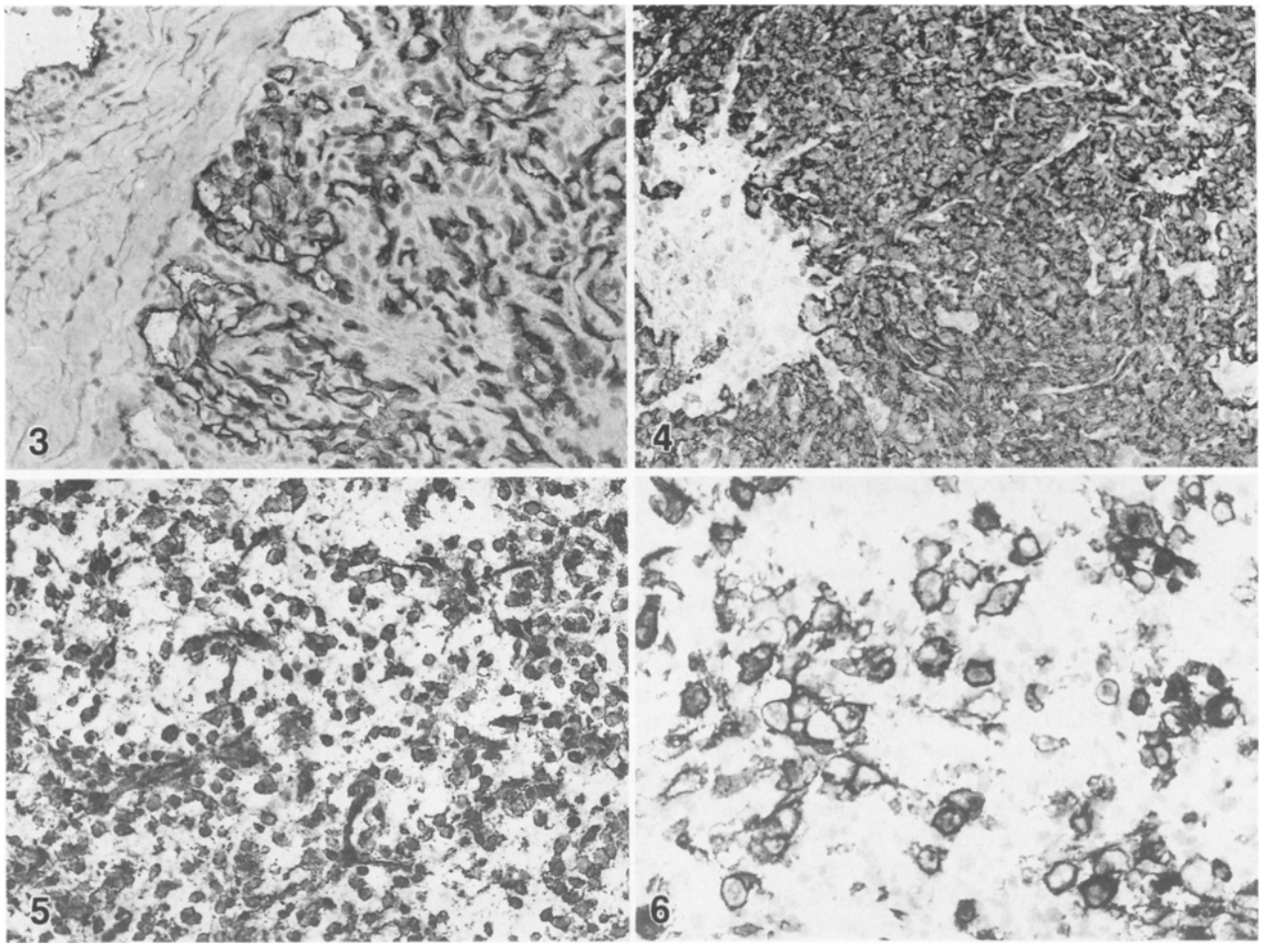


Fig. 3. Capillary haemangioma of the liver. Endothelial cells of proliferating capillaries are strongly CD34-positive detected by mAb My10. ×175

Fig. 4. Haemangiosarcoma of the liver. mAb My10 discloses strong expression of CD34 molecules in the entire tumour cell population. ×140

Fig. 5. Myxoid liposarcoma of the lower extremity stained with mAb OKM5. All tumour cells and endothelial cells of the plexiform capillary network are CD36-positive. The myxoid matrix is unstained. ×140

Fig. 6. Embryonal rhabdomyosarcoma of the retroperitoneum. CD36 recognized by mAb OKM5 is strongly expressed in/on rhabdomyoblasts while undifferentiated tumour cells lack any detectable CD36 molecules. ×175

CD36-positive (Fig. 6) as were skeletal muscle fibres microtopographically associated with tumour cells in 5 STT. In 1 of 12 malignant fibrous histiocytomas within the entire tumour cell population and in 1 case within a tumour cell subpopulation CD36 molecules were also expressed.

Comment. The CD36 molecule, also known as platelet gpIV(gpIIIb) antigen, is a primary adhesion receptor for collagen (Tandon et al. 1989). Outside the haematopoietic system, where it is mainly expressed by monocytes/macrophages and platelets, CD36 has been observed in some endothelial cells of capillaries and high endothelial venules and in adipocytes (von dem Borne et al. 1989). Thus, CD36 expression in endothelial cells of capillaries and in STT of vascular origin parallels that in their non-neoplastic counterparts. Unlike CD34, CD36 was also found in pericytic tumour cells of some haemangiopericytomas. CD36 expression in all lipomas and myxoid liposarcomas is in accordance with the non-neoplastic state. The frequent absence of CD36 in less differentiated tumours of adipose tissue indicates an abrogation/loss of this antigen in the course of malignant transformation. The data on CD36 expression in extrahaematopoietic mesenchymal cells could be enriched by the determination of CD36 expression in fetal and regenerative skeletal muscle cells and in some rhabdomyoblasts. Since normal adult skeletal muscle cells were only weakly and inconsistently CD36-positive, the over- and/or re-expression of CD36 in immature and regenerative skeletal muscle cells might indicate a role of this antigen in developmental processes. These findings parallel, although to a much lesser extent, data on the expression of CD56 molecules in skeletal muscle tissue (see below).

CD10 antigen

Non-neoplastic mesenchymal cells. Both thin and thick nerve fibres of peripheral nerves were at least weakly CD10-positive and this antigen was optional in fibrocytes/-blasts.

Soft tissue tumours. CD10 was consistently expressed in 3 of 4 leiomyomas and 9 of 16 leiomyosarcomas exhibiting spindle, round and pleomorphic cell differentiation in various amounts, and at least a tumour cell subset of 1 leiomyoma and 4 leiomyosarcomas (Fig. 7) and of 3 of 8 rhabdomyosarcomas expressed CD10 molecules. With respect to tumours of fibrous and synovial tissue, CD10 antigens were detectable in all tumour cells of 1 of 3 aggressive fibromatoses and 1 of 5 monophasic fibrous synovial sarcomas. Two aggressive fibromatoses, 2 of 4 fibrosarcomas and 2 monophasic fibrous synovial sarcomas showed CD10-positive and -negative neoplastic cells in various amounts. Concerning malignant fibrous histiocytomas, consistent CD10 expression was observed in 9 of 12 cases including 8 of pleomorphic and 1 of giant cell subtype (Fig. 8). One pleomorphic, 1 storiform and 1 giant cell malignant fibrous histiocytoma exhibited at least a CD10-positive tumour cell subset. In osteosarcomas, CD10 was consistently expressed in 1 of 4 cases; 3 osteosarcomas were at least partially CD10-positive. Regarding liposarcomas, consistent expression of CD10 was observed in 3 of 11 cases of myxoid, pleomorphic and round cell subtype, respectively, and 2 myxoid and 2 pleomorphic liposarcomas were at least partially CD10-positive. In tumours of the peripheral nervous system, only 1 of 14 malignant schwannomas were CD10-positive throughout; 1 of 3 neurilemomas and 3 malignant schwannomas, 2 of which showing focal rhabdomyoblastic differentiation (so-called Triton tumours), contained CD10-positive and -negative tumour cells in various amounts. One case of Ewing's sarcoma was consistently CD10-positive whereas 5 of 5 peripheral neuroepitheliomas, 9 of 9 (ganglio-)neuroblastomas, all tumours of vascular origin and morphologically well-defined tumours of uncertain histogenesis lacked any detectable CD10 molecules.

Comment. CD10 antigen, which for long has been known as common acute lymphoblastic leukaemia antigen (cALLa), has recently been shown to be identical to neutral endopeptidase (Letarte et al. 1988; Shipp et al. 1988). CD10 molecules are expressed in various normal and malignant extrahaematopoietic mesenchymal and neuroectodermal tissues. These include normal fibroblasts (Braun et al. 1983), breast myoepithelial cells (Metzgar et al. 1981; Gusterson et al. 1986) as well as gliomas (Monod et al. 1989), retinoblastomas (Seshadri et al. 1986), and malignant melanomas (Jongeneel et al. 1989). Furthermore, CD10 expression has been observed in malignant fibrous histiocytomas (Wood et al. 1986), in various other STT (Mechtersheimer et al. 1989) and in some small, round, blue cell sarcomas (Sugimoto et al. 1984; Pilkington and Pallesen 1989; Ebener et al. 1990). In this study, the knowledge of the distribution pattern in non-neoplastic cells was enriched by the detection of CD10 expression in both thick and thin nerve fibres of peripheral nerves. In the tumours of the peripheral nervous system, however, expression of CD10 was scarce. In contrast, but in line with their expression in normal fibrocytes/-blasts, CD10 molecules were detectable in the majority of fibrous tumours and of monophasic fibrous synovial sarcomas. What is more, CD10 expression in malignant fibrous histiocytomas could be strengthened in this study and was also found in all osteosarcomas. In line with data from Ebener et al. (1990), but in contrast to those of Pilkington and Pallesen (1989), all (ganglio-)neuroblastomas studied were CD10-negative throughout as were all peripheral neuroepitheliomas. Finally, the frequent expression of CD10 molecules in leiomyomas and leiomyosarcomas as well as their optional expression in rhabdomyosarcomas and in liposarcomas, and their absence in their non-neoplastic counterparts is worth mentioning. The fact that CD10 is identical to neutral endopeptidase, enzymatic activity of which has been demonstrated in CD10-positive malignant melanomas and gliomas (Jongeneel et al. 1989; Monod et al. 1989), might be of interest in view of the biological behaviour of CD10-positive STT.

CD13 antigen

Non-neoplastic mesenchymal cells. Osteoclasts were consistently CD13-positive (Fig. 9). CD13 molecules were also detectable in the majority of fibrocytes/-blasts, especially in those surrounding blood vessels and cutaneous appendages and were present in some scattered histiocytes.

Soft tissue tumours. CD13 molecules were consistently expressed in 1 leiomyoblastoma and 3 of 16 leiomyosarcomas, including osteoclast-like giant cells in 1 case. Two of four leiomyomas and 8 of 16 leiomyosarcomas showed CD13-positive and -negative neoplastic cells in various amounts, and 1 of 3 aggressive fibromatoses and 1 of 5 monophasic fibrous synovial sarcomas were CD13-positive throughout (Fig. 10). In addition, CD13 molecules were found in a tumour cell subpopulation of 2 aggressive fibromatoses, 2 fibrosarcomas and 1 monophasic synovial sarcoma while lacking in 2 of 2 fibromas. In malignant fibrous histiocytomas, consistent expression of CD13 was found in 2 cases; 7 tumours expressed CD13 molecules in various amounts including CD13-positive osteoclast-like giant cells present in 2 cases. CD13 molecules were also identified in all tumour cells of 1 of 4 osteosarcomas and in at least a minor tumour cell subset in 3 osteosarcomas; 3 of 3 chondrosarcomas lacked any detectable CD13 molecules. Regarding the tumours of the peripheral nervous system, the CD13 antigen was consistently expressed in 1 of 14 malignant schwannomas; 4 malignant schwannomas displayed CD13-positive and -negative tumour cells in various amounts. Two of 11 liposarcomas, 1 of pleomorphic and 1 of sclerosing subtype, were consistently and 1 myxoid, inflammatory and pleomorphic liposarco-

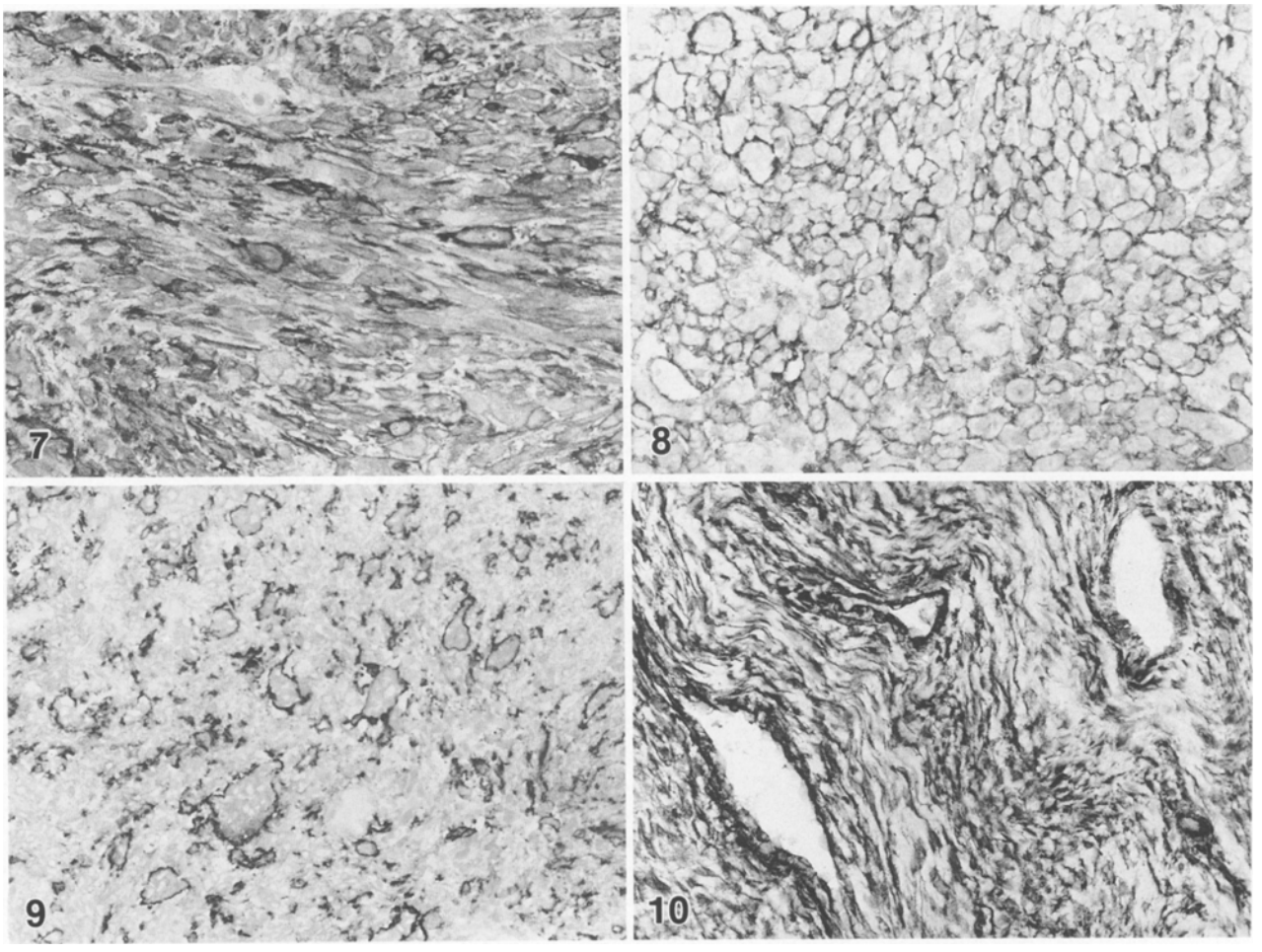


Fig. 7. Pleomorphic leiomyosarcoma of the retroperitoneum. Immunostaining with mAb J5 reveals CD10 expression in a major tumour cell subpopulation. ×175

Fig. 8. Pleomorphic malignant fibrous histiocytoma of the upper extremity stained with mAb J5. Nearly all tumour cells exhibit strong surface staining for CD10 molecules. ×175

Fig. 9. Ossifying myositis of the vertebral column. mAb My7 determines membrane-bound positivity of osteoclasts and osteoclast-like giant cells while intermingled fibroblasts are CD13-negative. ×224

Fig. 10. Aggressive fibromatosis of the mesenterial radix. The tumour cells which are arranged in ill-defined fascicles are CD13-positive throughout visualized by mAb My7. ×140

ma, respectively, were partially CD13-positive. Some undifferentiated tumour cells of an Ewing's sarcoma expressed CD13 molecules as well. As was the case with CD10, all tumours of the autonomic nervous system, of vascular tissue and, except for a partially CD13-positive epithelioid sarcoma, the neoplasms of uncertain histogenesis lacked any detectable CD13 molecules.

Comment. The CD13 molecule, which is considered a pan-myeloid antigen (Griffin et al. 1981), has been proven to be identical to aminopeptidase N, thus belonging, as CD10, to the same metalloprotease family (Look et al. 1989). In normal extrahaematopoietic tissue, CD13 has been determined in fibroblasts and in osteoclasts (Hogg and Horton 1987); these results were confirmed in this study. In neoplasms, it has been detected in both the mononuclear and the multinucleated giant cells of a benign giant cell tumour of tendon sheath (Wood et al. 1988). In accordance with these findings and with previous data on CD13 expression in STT (Mechtersheimer et al. 1990a), CD13 antigens in the present study were found in osteoclast-like giant cells included in 2 malignant fibrous histiocytomas and in 1 leiomyosarcoma. A subset of mononuclear tumour cells in some malignant fibrous histiocytomas expressed also CD13 antigens, as did osteoclastic and even spindle tumour cells in osteosarcomas. Based on the observations for CD13 positivity and, to a much lesser extent, for CD36 positivity in various STT, the optional expression of these antigens alone cannot be considered a strong argument in favour of a true histiocytic origin of malignant fibrous histiocytoma. Nevertheless, it should be noted that our results are in agreement with those of Strauchen and Dimitriou-Bona (1986), who demonstrated positivity for monocyte/macrophage antigens in malignant fibrous histiocytomas, whereas Roholl et al. (1985) and Lawson et al.

(1987) did not find expression of any of these antigens in malignant fibrous histiocytomas. Despite their complete absence in normal smooth muscle cells, peripheral nerves and adipocytes, some tumours derived from these cell types also expressed CD13 molecules. The significance of CD13 expression in these neoplasms cannot be explained, but it should be remembered that CD13, like CD10, may display enzymatic activity in these tumours, thus influencing their biological behaviour. Lastly, the complete absence of CD13 molecules in the vast majority of highly malignant, small, round, blue cell sarcomas is a noteworthy feature. All in all, the detection of CD13 expression in a great variety of STT suggests this antigen to be valuable for functional analysis rather than for purely descriptive immunomorphology of STT.

Comparison between CD10 and CD13 expression. The comparison between the expression pattern in STT of CD10 and CD13 molecules, which both belong to the metalloprotease family, disclosed a co-expression in 2 of 4 leiomyomas, 7 of 16 leiomyosarcomas, 3 of 3 aggressive fibromatoses, 1 of 4 fibrosarcomas, 1 of 5 monophasic fibrous synovial sarcomas, 8 of 12 malignant fibrous histiocytomas, 3 of 4 osteosarcomas, 3 of 14 malignant schwannomas, and 1 Ewing's sarcoma. Thus, given the overall expression of CD10 and CD13 in STT, the co-expression of these antigens in tumours of smooth muscle, fibrous, fibro-histiocytic and osteogenic origin merits particular attention.

CD57 antigen

Non-neoplastic mesenchymal cells. CD57 molecules were restricted to thick nerve fibres of peripheral nerve trunks (Fig. 11) and to a major subpopulation of chromaffin cells of the adrenal medulla (Fig. 12).

Soft tissue tumours. Like in the non-neoplastic state, CD57 antigens were only rarely found in STT. A minor tumour cell subpopulation of 1 of 3 neurilemomas, 2 of 14 malignant schwannomas and 2 of 5 ganglioneuroblastomas expressed CD57 molecules (Fig. 13), as did a Schwann cell subset of 4 of 4 ganglioneuromas. In most STT studied, the presence of scattered CD57-positive lymphoid cells or some entrapped CD57-positive nerve fibres served as positive intrinsic control of the immune reaction ruling out false negative staining.

Comment. mAb Leu-7 was raised against the human T cell line HSB-2 and has been shown to recognize a differentiation antigen designated CD57, which is present on 15–20% of normal peripheral blood mononuclear cells containing most of the natural killer (NK) activity of the peripheral blood (Abo and Balch 1981). McGarry et al. (1983) demonstrated that CD57 mAb also recognize the myelin-associated glycoprotein (MAG), and found CD57 expression in myelinated nerve fibres of both the central and the peripheral nervous system. Furthermore, CD57 mAb has been proven to detect a sulphated carbohydrate epitope expressed on a portion of neural-cell adhesion molecule (N-CAM) isoforms (Kruse et al. 1984). Consequently, expression of CD57 has been observed in a series of neural and neuroectodermal tumours, including neurofibromas and neurofibrosarcomas (Swanson et al. 1987), malignant melanomas (Lipinski et al. 1983), malignant peripheral neuroectodermal tumours (Llombard-Bosch et al. 1988), Ewing's sarcomas (Pinto et al. 1989), small cell lung carcinomas and other neuroendocrine tumours (Michels et al. 1987) and even in rare leiomyosarcomas and monophasic fibrous synovial sarcomas (Swanson et al. 1987). This study not only confirms CD57 expression in thick, myelinated nerve fibres of peripheral nerves, it also shows expression of CD57 molecules in the majority of chromaffin cells of the adrenal medulla. In neoplasms, expression of CD57 was an extremely rare phenomenon, in contrast with the data reported in the literature. Since, in our hands, some CD57-positive lymphoid cells and/or thick nerve fibres showed reliable immune reaction and since mAb Leu-7 is of IgM isotype, the considerable number of CD57-positive STT reported in the literature might at least partially be the result of non-specific isotype effects which can be found in several epithelial cell types (personal observations, data not shown). It might also be possible that at least some cases of the CD57 positivity reported by other authors might be confined to entrapped non-neoplastic nerve fibres or lympho-histiocytic stromal cells. The latter, in this study, were discriminated from neoplastic cells by incubating one of the serial sections of each case with CD53 mAb HD77. All in all, the low frequency of CD57 expression in neural tumours compared with that of normal neural cells might reflect an abrogation/loss of this antigen in the course of neoplastic transformation. From the differential diagnostic point of view it is worth mentioning that CD57, although only rarely found in STT, was solely restricted to tumours of neural tissue.

CD56 antigen

Non-neoplastic mesenchymal cells. CD56 antigen was strongly and consistently expressed by thin but not by thick nerve fibres of peripheral nerve trunks, by satellite cells and on the surface of ganglion cells and by chromafin cells of the adrenal medulla. Cardiac muscle cells were strongly CD56-positive throughout, as were fetal and the majority of regenerative adult skeletal muscle cells. Normal adult skeletal muscle cells, however, were CD56-negative except for scattered muscle fibres of eye muscles and muscles of the tongue. Smooth muscle cells of visceral organs, uterus and prostate were at least partially and/or weakly CD56-positive whereas those of vessels walls lacked any detectable CD56 molecules.

Soft tissue tumours. CD56 molecules were strongly and consistently expressed in 2 of 4 leiomyomas, 1 leiomyoblastoma, 9 of 16 leiomyosarcomas and 6 of 8 rhabdomyosarcomas (Fig. 14). CD56 molecules were also expressed in at least a minor tumour cell subset of 1 leiomyoma, 1 leiomyosarcoma and 2 rhabdomyosarcomas

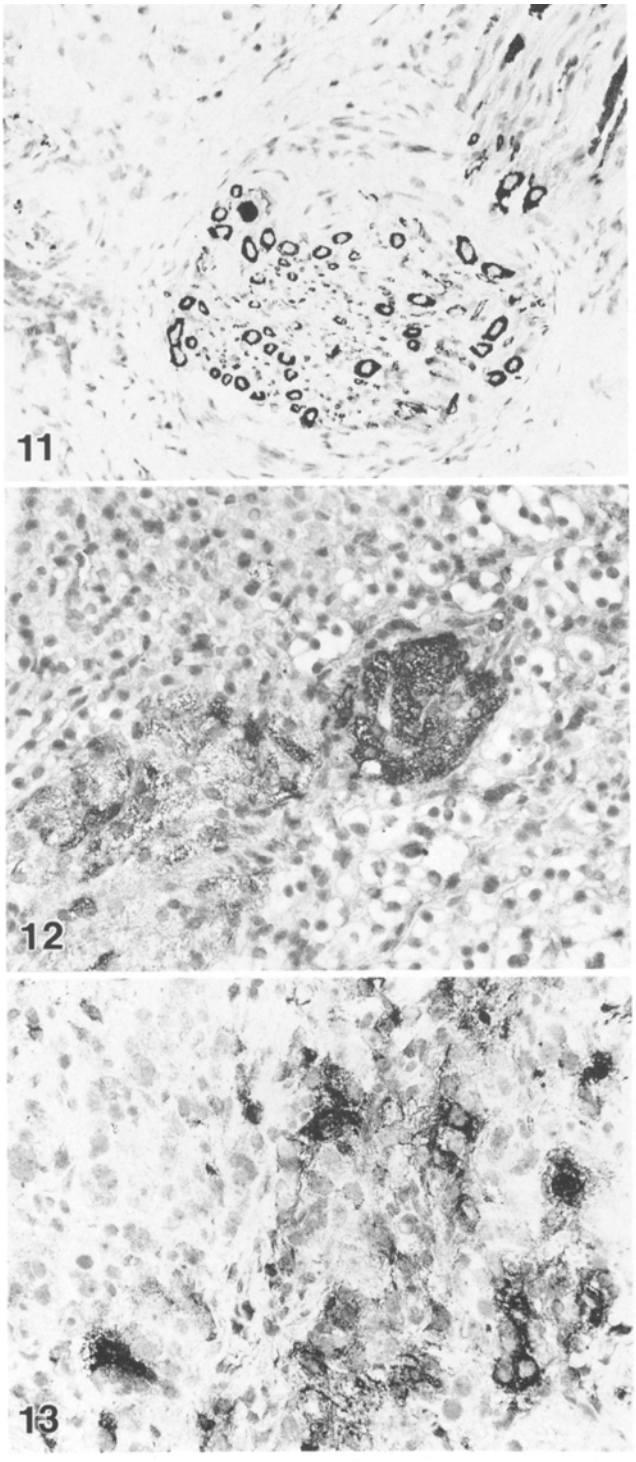


Fig. 11. Granular cell tumour of the upper extremity including a minor peripheral nerve trunk of adjoining skin infiltrated by the tumour. CD57 detected by mAb Leu-7 is strongly expressed in myelin sheaths of thick but not of thin nerve fibres. Polygonal tumor cells are CD57-negative throughout. × 180

Fig. 12. Normal adrenal gland. mAb Leu-7 discloses expression of CD57 molecules in the majority of medullary chromaffin cells. Adrenal cortex lacks any detectable CD57 molecules. × 224

Fig. 13. Ganglioneuroblastoma of the thoracic wall. Expression of CD57 antigens visualized by mAb Leu-7 is confined to a minor tumour cell subpopulation. × 224

and in all tumour cells of 1 of 2 fibromas, 3 of 4 fibrosarcomas (Fig. 15), 3 of 5 monophasic fibrous synovial sarcomas, 1 of 12 malignant fibrous histiocytomas and 1 of 4 osteosarcomas as well as in a tumour cell subset of 1 fibroma, 3 aggressive fibromatoses, 4 malignant fibrous histiocytomas, 3 osteosarcomas and 2 of 3 chondrosarcomas. In tumours of the peripheral nervous system, 2 of 3 neurilemomas and 4 of 14 malignant schwannomas exhibited strong and consistent expression of CD56 molecules; 1 neurilemoma, 1 granular cell tumour and 6 malignant schwannomas comprised CD56-positive and -negative tumour cells in various amounts while 4 malignant schwannomas lacked any detectable CD56 molecules. Likewise, 4 of 5 peripheral neuroepitheliomas and 1 Ewing's sarcoma were CD56-negative throughout; 1 peripheral neuroepithelioma comprised only scattered CD56-positive tumour cells. A striking feature was the consistent expression of CD56 molecules in 5 of 5 ganglioneuroblastomas and in 4 of 4 neuroblastomas as well as in the majority of the Schwann cell compartments and on the surface of ganglion cells of 4 of 4 ganglioneuromas (Fig. 16). In some tumours of autonomic ganglia and in some rhabdomyosarcomas, CD56 exhibited a granular and even an extracellular pattern of expression, suggesting some shedding of CD56 molecules. Finally, at least a tumour cell subset of 5 of 11 liposarcomas, 2 of 6 haemangiopericytomas and 1 epithelioid sarcoma was CD56-positive.

Comment. Like CD57, the CD56 molecule is a well-known NK cell antigen (Lanier et al. 1986). Recently, Lanier et al. (1989) found CD56 also expressed in neural tissue and were able to demonstrate its identity to the 140 kDa isoform of N-CAM. CD56 expression has also been observed in neuroblastomas, Ewing's sarcomas and rhabdomyosarcomas (Feickert et al. 1989; Mechtersheimer et al. 1991). The consistent expression of CD56 found in all (ganglio-)neuroblastomas examined in this study contrasts with data by Feickert et al. (1989) who, by application of the same CD56 mAb, determined CD56 expression in only 6 of 11 neuroblastomas. Further evidence for broad N-CAM expression in neuroblastomas, however, is provided by Lipinski et al. (1987), using a rabbit anti-human N-CAM antiserum and by mAb of small cell lung cancer and neuroblastoma workshops which also share an epitope in common with N-CAM (Patel et al. 1989a, b). The frequent presence of CD56 in (ganglio-)neuroblastomas contrasts with its extremely rare expression in peripheral neuroepitheliomas. From these data it is suggested that CD56 might be a useful tool for discriminating between these subtypes of small, round, blue cell sarcoma. Epitopes detected by mAb of the small cell lung cancer and neuroblastoma workshops as well as CD56 molecules have also been found in rhabdomyosarcomas (Patel et al. 1989a, b; Feickert et al. 1989). In line with these data, we identified expression of CD56 in all rhabdomyosarcomas studied. Since, in the normal state, N-CAM is present in fetal but absent in normal adult skeletal muscle cells and re-expressed in rhabdomyosarcomas and regenerative skeletal muscle cells, we suggest that, in skeletal

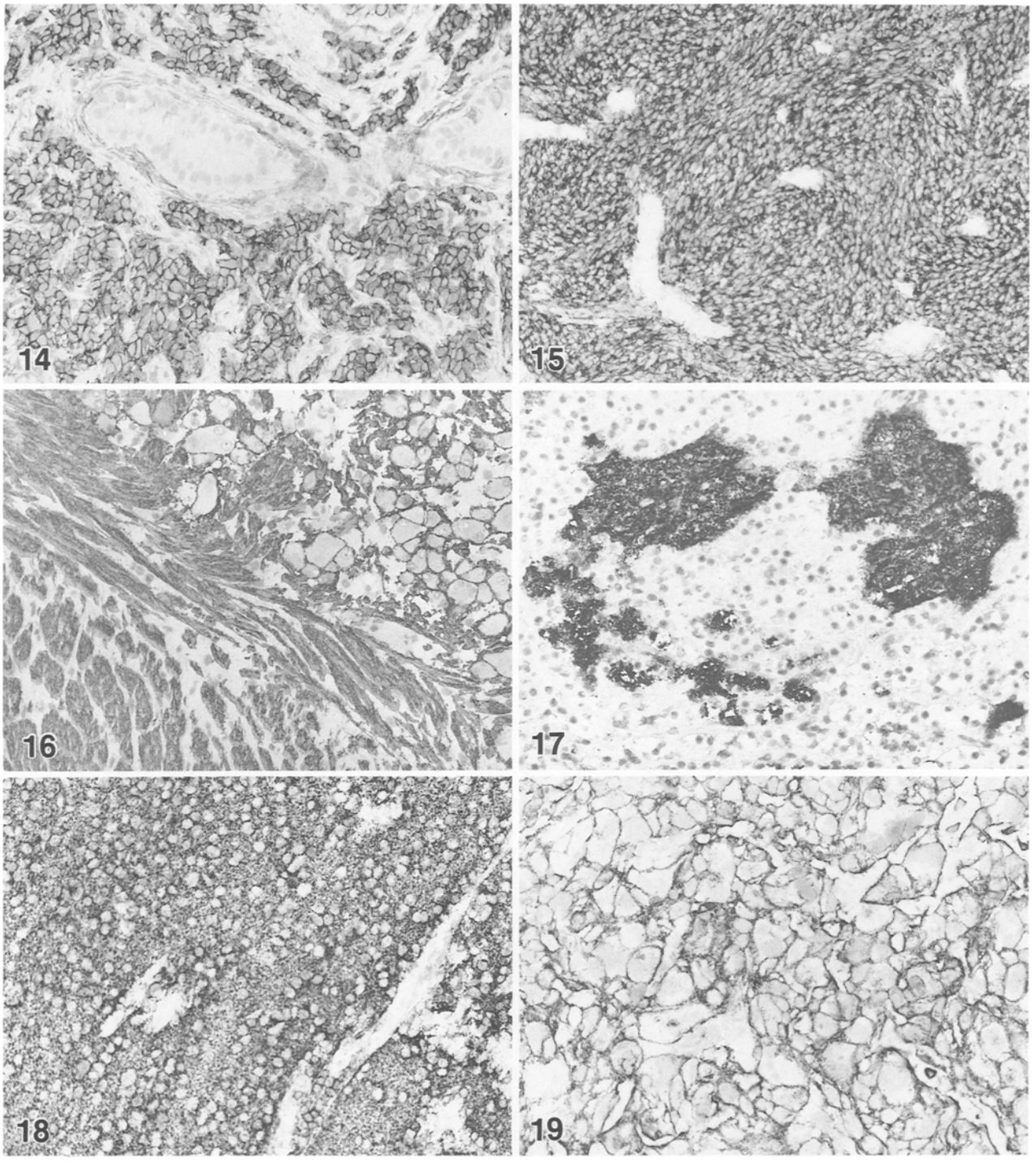


Fig. 14. Testicular rhabdomyosarcoma exclusively composed of small, round, undifferentiated tumour cells. The neoplastic cells exhibit strong and consistent staining for CD56 molecules detected by mAb Leu-19. Seminiferous tubules are CD56-negative. ×175

Fig. 15. Fibrosarcoma of the scapular region stained with mAb Leu-19. The tumour cell population is strongly CD56-positive throughout. Endothelial cells of intermingled blood vessels are CD56-negative. ×140

Fig. 16. Ganglioneuroma of the retroperitoneum. CD56 visualized by mAb Leu-19 is strongly expressed on the surface of ganglion cells and on the entire Schwann cell population. ×140

Fig. 17. Normal adrenal gland. mAb OKB2 displays strong and consistent CD24 expression in medullary chromaffin cells. Adrenal cortex is CD24-negative throughout. ×175

Fig. 18. Neuroblastoma of the adrenal gland. mAb OKB2 discloses granular CD24 expression of the undifferentiated tumour cells and, in addition, reveals even extracellularly localized CD24 antigen. ×175

Fig. 19. Osteoblastic osteosarcoma of the lower extremity stained with mAb OKB2. The tumour cells exhibit strong and consistent membrane-bound staining for CD24 molecules. ×175

muscle tissue, this antigen be considered an onco-developmental antigen. Comparable results have been obtained in renal tissue (Roth et al. 1988). Expression of CD56, however, was not restricted to neural and skeletal (and cardiac) muscle tissue. CD56 molecules were frequently expressed in leiomyomas and leiomyosarcomas as they are (weakly) in some normal smooth muscle cells. Transient expression of N-CAM has also been detected in smooth muscle cells of rat aortic muscle walls both in situ and in vitro (Akeson et al. 1988). The expression of CD56 in a tumour cell subpopulation of some liposarcomas, fibrosarcomas, osteosarcomas and, although only sporadically, in malignant fibrous histiocytomas is opposed to the complete absence of CD56 in their non-neoplastic counterparts. This neo-expression of CD56 antigens in STT seems paradoxical since it is suggested that low amounts or even absence of N-CAM might lead to a loss of contact inhibition allowing for uncontrolled cell detachment. This view is backed by the observation that the metastatic melanoma cell line K1735-M1 expressed less N-CAM than did the non-metastazising K1735-C116 line (Linnemann et al. 1989), and by the fact that mAb MUC18, a marker for tumour progression in human melanoma, has been shown to display sequence similarity to N-CAM (Lehmann et al. 1989). The fact that a considerable number of malignant schwannomas exhibited at least partial or even complete absence of CD56 molecules, though, might be explained by this hypothesis. In summary, the CD56 pattern in STT might reflect a special functional state of the neoplastic cells which, at present, cannot be further specified. From the viewpoint of diagnostics, the differential expression of CD56 in subtypes of the small, round, blue cell sarcoma category merits special attention.

CD24 antigen

Non-neoplastic mesenchymal cells. Satellite cells of ganglion cells and chromaffin cells of the adrenal medulla showed consistent and sometimes granular expression of CD24 molecules (Fig. 17); thin nerve fibres were also CD24-positive.

Soft tissue tumours. A striking feature was the consistent CD24 expression in 5 of 5 ganglioneuroblastomas and in 4 of 4 neuroblastomas (Fig. 18), whereas in peripheral neuroepitheliomas this antigen was restricted to a minor tumour cell subpopulation of 1 of 5 cases; 1 Ewing's sarcoma was also CD24-negative. Moreover, CD24 molecules were found in all neoplastic cells of 3 of 16 leiomyosarcomas, 2 of 14 malignant schwannomas, 2 of 11 liposarcomas and 1 of 4 osteosarcomas (Fig. 19) as well as in a tumour cell subset of 2 malignant schwannomas, 4 liposarcomas, 1 of 5 monophasic fibrous synovial sarcomas, 1 of 12 malignant fibrous histiocytomas, 1 of 3 chondrosarcomas and 3 osteosarcomas. Like CD56 molecules, CD24 antibodies showed a granular staining pattern and sometimes an even extracellularly localized antigen (Fig. 18), suggesting the existence of some shedding mechanism in certain cell types (or tumours).

Comment. CD24, which comprises at least three different epitopes and which is expressed on B lineage cells and granulocytes (Clark et al. 1989), is not restricted to haematopoietic tissue. Some CD24 epitopes are expressed on vascular endothelial cells (Nadler et al. 1986), and CD24 molecules have been observed in neuroblastoma cells (Sugimoto et al. 1984; Stockinger et al. 1987; Ebener et al. 1990). This study adds further evidence for the expression of CD24 molecules in neural tissues. In line with data reported in the literature (see above) and in accordance with their presence in chromaffin cells of the adrenal medulla, we found consistent expression of CD24 antigens in all (ganglio-)neuroblastomas studied. Since, in contrast, all rhabdomyosarcomas, an Ewing's sarcoma and the majority of peripheral neuroepitheliomas studied lacked any detectable CD24 molecules, CD24 proved to be of great value in the differential diagnosis of small, round, blue cell sarcomas. Although CD24 was expressed in thin nerve fibres, tumours of the peripheral nervous system only rarely expressed CD24 molecules, suggesting an abrogation/loss of CD24 molecules during malignant transformation of peripheral nerves while all osteosarcomas contained at least a CD24-positive tumour cell subset although osteoblasts were CD24-negative throughout.

Comparison between CD56 and CD24 expression. The comparison between CD56 and CD24 expression in STT disclosed a consistent coexpression of these molecules in all (ganglio-)neuroblastomas studied whereas the peripheral neuroepitheliomas and the Ewing's sarcoma studied lacked any detectable CD56 and CD24 molecules, excepting a single case of peripheral neuroepithelioma which showed co-expression of CD56 and CD24 in a minor tumour cell subpopulation. The consistent absence of CD24 molecules in all rhabdomyosarcomas examined is another noteworthy feature since all these tumours were more or less CD56-positive.

MHC class I and class II/Ii antigens

HLA-A,B,C/β_2m antigens

Non-neoplastic mesenchymal cells. Strong and consistent expression of HLA-A,B,C/β_2m molecules was determined in fibrocytes/-blasts, histiocytes, osteoblasts, osteoclasts, thin nerve fibres of peripheral nerve trunks, chromaffin cells of adult but not of fetal adrenal medulla and in endothelial cells of blood vessels. Lymph vessel endothelium, however, was HLA-A,B,C/β_2m-negative, and smooth muscle cells of the uterus and prostate were but weakly HLA-A,B,C/β_2m-positive. Smooth muscle cells of vessel walls, skeletal and cardiac muscle cells, chondrocytes, thick nerve fibres, ganglion cells and chromaffin cells of fetal adrenal medulla lacked any detectable HLA-A,B,C/β_2m molecules.

Soft tissue tumours. Strong and consistent expression of HLA-A,B,C/β_2m antigens was found in 4 of 4 leiomyomas, 1 leiomyoblastoma, 9 of 16 leiomyosarcomas

Table 5. Expression of HLA-A,B,C/β_2m in neoplastic cells of STT

Phenotype	n	+	(+)	+/−	−
Leiomyoma	4	4	–	–	–
Leiomyoblastoma	1	1	–	–	–
Leiomyosarcoma	16	8	4	2	2
Rhabdomyosarcoma	8	1	–	5	2
Fibroma	2	–	–	–	2
Aggressive fibromatosis	3	3	–	–	–
Fibrosarcoma	4	1	–	1	2
Synovial sarcoma	5	1	–	2	2
Malignant fibrous histiocytoma	12	10	–	2	–
Chondrosarcoma	3	1	–	2	–
Osteosarcoma	4	4	–	–	–
Neurilemoma	3	3	–	–	–
Granular cell tumour	1	1	–	–	–
Malignant schwannoma	14	8	1	3	2
Peripheral neuroepithelioma	5	3	1	1	–
Ewing's sarcoma	1	1	–	–	–
Ganglioneuroma	4GC	–	–	–	4
	SC	4	–	–	–
Ganglioneuroblastoma	5GC	–	–	–	5
	NB	–	–	2	3
Neuroblastoma	4	–	–	–	4
Lipoma	2	–	–	–	2
Liposarcoma	11	2	2	3	4
Lymphangioma	1EC	1	–	–	–
	LE	–	–	–	1
Haemangioma	4EC	4	–	–	–
Haemangiopericytoma	6	5	–	1	–
Haemangiosarcoma	1	1	–	–	–
Epithelioid sarcoma	1	1	–	–	–
Alveolar soft part sarcoma	1	1	–	–	–
Clear cell sarcoma	2	2	–	–	–

GC, Ganglion cells; SC, Schwann cells; NB, neuroblasts; EC, vascular endothelial cells; LE, lymphatic endothelial cells

(Fig. 20) and 1 of 8 rhabdomyosarcomas which was exclusively composed of oval or rounded rhabdomyoblasts. Three leiomyosarcomas were weakly HLA-A,B,C/β_2m-positive throughout, 2 cases comprised HLA-A,B,C/β_2m-positive and -negative tumour cells in various amounts. Compared to the situation found in their non-neoplastic counterparts, the expression of HLA-A,B,C/β_2m antigens in these tumours has at least partly to be considered an aberrant induction/neo-expression. In addition, in 5 further rhabdomyosarcomas, some rounded or elongated rhabdomyoblasts showed an aberrant induction of HLA-A,B,C/β_2m molecules (Fig. 21). A neo-expression of HLA-A,B,C/β_2m antigens was also found in the entire neoplastic population of 1 of 3 chondrosarcomas (Fig. 22) and in 4 of 11 liposarcomas (Fig. 23) and in a minor tumour cell subset of 2 chondrosarcomas and 3 liposarcomas. In line with the situation found in non-neoplastic mesenchymal cells, 3 of 3 aggressive fibromatoses, 1 of 4 fibrosarcomas, 1 of 5 monophasic fibrous synovial sarcomas, 10 of 12 malignant fibrous histiocytomas and 4 of 4 osteosarcomas were strongly HLA-A,B,C/β_2m-positive throughout. However, 1 fibrosarcoma, 2 monophasic fibrous synovial sarcomas and 2 malignant fibrous histiocytomas were at least partially HLA-A,B,C/β_2m-negative; 2 of 2 fibromas, 2 fibrosarcomas and 2 monophasic fibrous synovial sarcomas were completely devoid of any detectable HLA-A,B,C/β_2m molecules (Fig. 24). These findings indicate an aberrant abrogation/loss of HLA-A,B,C/β_2m antigens in a considerable number of tumours of fibrous origin. It is interesting to note that consistent and strong expression of HLA-A,B,C/β_2m molecules was found in 3 of 5 peripheral neuroepitheliomas and in 1 Ewing's sarcoma. One further peripheral neuroepithelioma was consistently but weakly HLA-A,B,C/β_2m-positive, 1 case displayed HLA-A,B,C/β_2m-positive and -negative tumour cells in various amounts, but 4 of 4 neuroblastomas and 3 of 5 ganglioneuroblastomas lacked any detectable HLA-A,B,C/β_2m molecules. These data are in accordance with the absence of HLA-A,B,C/β_2m molecules in chromaffin cells of fetal adrenal medulla. In 2 ganglioneuroblastomas, on the other hand, an aberrant neo-expression of these molecules was found in a minor tumour cell subset. The expression of HLA-A,B,C/β_2m antigens in endothelial cells of proliferating blood vessels in 4 of 4 haemangiomas, in the neoplastic population of 6 of 6 haemangiopericytomas and 1 haemangiosarcoma as well as the absence of these molecules in endothelial cells of proliferating lymph vessels in 1 lymphangioma has to be considered orthologous with respect to the data reported for their non-neoplastic counterparts. Lastly, strong and consistent expression of HLA-A,B,C/β_2m determinants was observed in 1 epithelioid sarcoma, 1 alveolar soft part sarcoma and 2 of 2 clear cell sarcomas of tendons and aponeuroses.

HLA-D/Ii antigens

Non-neoplastic mesenchymal cells. Consistent expression of the HLA-D sublocus products HLA-DR, -DP and -DQ as well as of Ii was found in histiocytes and the so-called DIC; thin nerve fibres expressed optionally HLA-DR and -DP antigens in the absence of Ii and HLA-DQ; endothelial cells, in addition, were sometimes HLA-DR-positive but lacked Ii, HLA-DP and -DQ. The great majority of non-neoplastic mesenchymal cells, however, was completely HLA-D/Ii-negative.

Soft tissue tumours. Corresponding to their restricted expression patterns in non-neoplastic mesenchymal cells, HLA-D/Ii determinants were rarely found in STT. The expression of HLA-DR, -DP, -DQ and Ii determinants identified in a neoplastic subset of 4 of 12 malignant fibrous histiocytomas, respectively, might, from the histogenetic point of view, reflect an orthologous situation, whereas the expression of these molecules in a tumour cell subset of 2 of 4 osteosarcomas is aberrant with respect to the complete absence of these molecules in osteoclasts and -blasts. Furthermore, 1 of 4 leiomyomas, 1 of 16 leiomyosarcomas, and 1 of 6 haemangiopericytomas showed aberrant induction/neo-expression of at least 1 of the HLA-D/Ii molecules in a tumour cell subpopulation, as did some neoplastic cells and a Schwann cell subpopulation in 2 of 3 neurilemomas and 4 of 4 ganglioneuromas (Fig. 25), respectively. Once

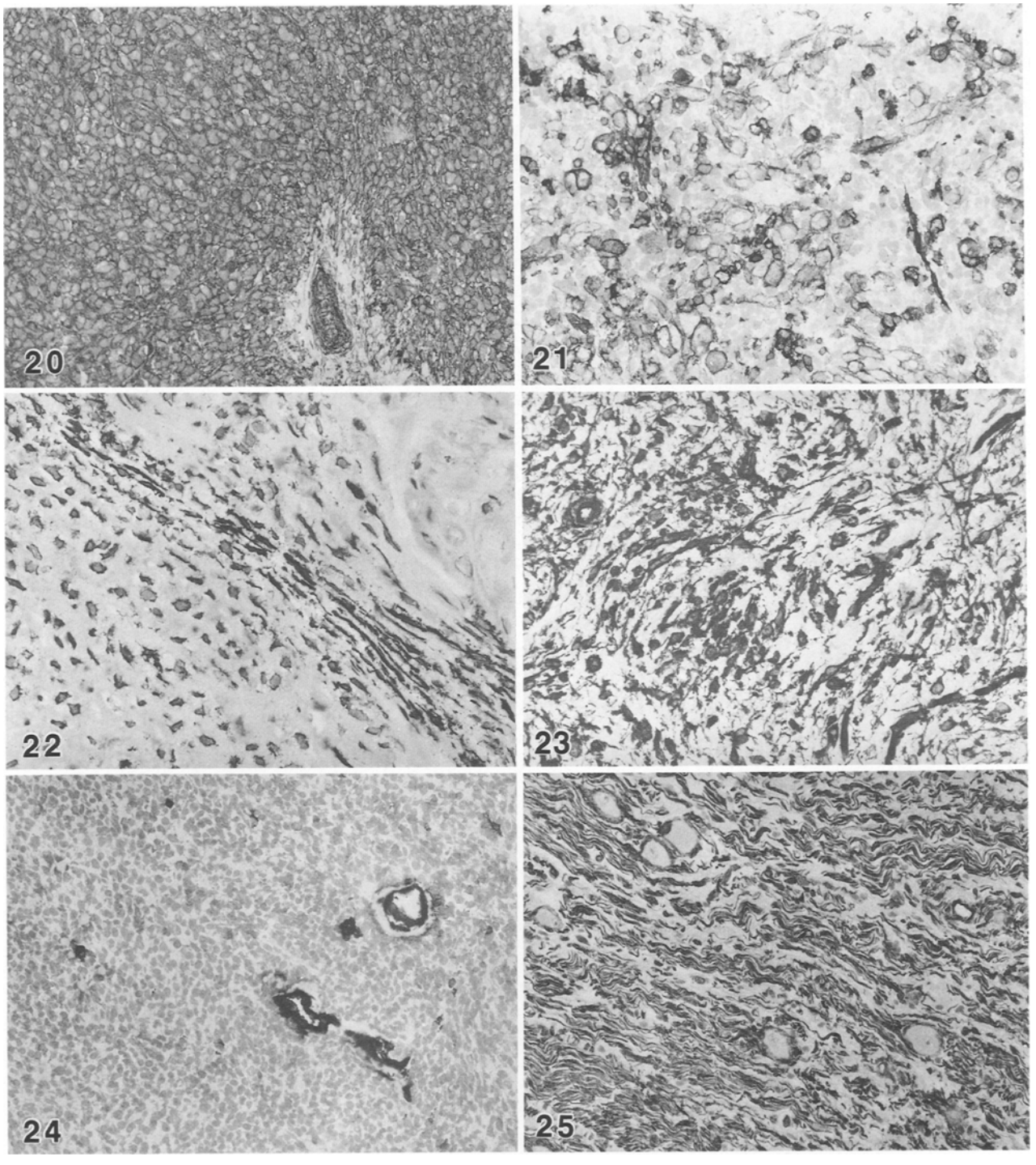


Fig. 20. Leiomyosarcoma of the femoral vein stained with mAb W6/32. The tumour cells are strongly and consistently HLA-A,B,C-positive. × 140

Fig. 21. Embryonal rhabdomyosarcoma of the retroperitoneum. β_2m molecules visualized by mAb BBM.1 are strongly expressed in/on rhabdomyoblasts while undifferentiated tumour cells lack any detectable β_2m antigens. × 140

Fig. 22. Chondrosarcoma of the rib. Expression of HLA-A,B,C antigens detected by mAb W6/32 is determined in/on undifferentiated chondroid tumour cells; more differentiated neoplastic cells remain HLA-A,B,C-negative. × 175

Fig. 23. Myxoid liposarcoma of the lower extremity. mAb BBM.1 reveals strong β_2m expression in all tumour cells and in endothelial cells of capillaries. × 175

Fig. 24. Monophasic fibrous synovial sarcoma of the lower extremity stained with mAb W6/32. The neoplastic population is completely HLA-A,B,C-negative. HLA-A,B,C-positive endothelial cells serve as positive intrinsic control for the immune reaction. × 140

Fig. 25. Ganglioneuroma of the retroperitoneum. mAb ISCR3 illustrates strong HLA-DR expression by the Schwann cell compartment and by scattered satellite cells of ganglion cells the perika-ryons of which are HLA-DR-negative. × 175

again, these HLA-D/Ii-positive tumour cells could be discriminated from DIC by absence of CD53 molecules. Also, in 1 of 2 clear cell sarcomas of tendons and aponeuroses a minor tumour cell population was Ii-, HLA-DR-, -DP- and -DQ-positive. Interestingly, these HLA-D/Ii-positive tumour cells were situated in the vicinity of a localized inflammatory infiltrate.

Comment. Class I (HLA-A,B,C)- and class II (HLA-DR, -DP and -DQ)-antigens of the MHC serve mainly as restriction elements of the cellular immune response by interacting with the T cell antigen receptor of autologous T cells (reviewed by Krensky et al. 1990). Under normal conditions, HLA-A,B,C antigens are constitutively expressed in a large number of cell types while constitutive expression of HLA-D/Ii molecules is restricted to mature B lymphocytes, histiocytes, DIC and some epithelial cell types (Daar et al. 1984a, b; Natali et al. 1984). In many cell types, both class I and class II antigens are supplementarily regulated by mediators such as interferon-γ and tumour necrosis factor (Collins et al. 1984, 1986) and could in situ be observed during inflammation (e.g. Koretz et al. 1987). Aberrant induction or abrogation of these molecules has been found during malignant transformation (e.g. Momburg et al. 1986; Möller et al. 1987; Koretz et al. 1989; Mechtersheimer et al. 1990b).

In contrast with data in the literature which consider HLA-A,B,C/β_2m molecules to be widely distributed in non-neoplastic mesenchymal cells (Daar et al. 1984; Natali et al. 1984), this study revealed complete absence of these molecules in a considerable number of normal mesenchymal cell types. Compared with the data for their non-neoplastic counterparts, however, all tumours of smooth muscle origin, the majority of tumours of peripheral nerve sheath origin, and at least a tumour cell subpopulation of chondrosarcomas and some liposarcomas exhibited an aberrant induction/neo-expression of HLA-A,B,C/β_2m molecules. The absence of HLA-A,B,C/β_2m antigens in the majority of (ganglio-)neuroblastomas is in aggreement with data by various authors (Lampson et al. 1983; Whelan et al. 1985), although some ganglioneuroblastomas showed an aberrant induction of these molecules in at least a minor tumour cell population. Likewise, expression of HLA-A,B,C/β_2m molecules in rhabdomyoblasts has to be considered aberrant with respect to normal striated muscle cells. Honda and Rostami (1989) identified constitutive expression of class I antigens in myoblasts, which disappeared as myoblasts fused into multinucleated myotubes, thus suggesting a role of these molecules in cell recognition and interactions in myogenesis. Abnormal abrogation/loss of HLA-A,B,C/β_2m antigens in STT when compared with their normal counterparts was restricted to tumours of fibrous origin. Thus, considerable alterations in MHC class I antigen expression occur in STT which, in contrast to most carcinomas and malignant melanomas, consist mainly of an induction/neo-expression of these molecules. These aberrant changes of MHC class I antigens are suggested to be indicative of malignant transformation of mesenchymal cells.

Alterations in HLA-D/Ii expression were only rarely observed in STT. The presence of HLA-D/Ii antigens in some tumours of smooth muscle, vascular, and peripheral nerve sheath origin must, at least partly, be considered aberrant with respect to the non-neoplastic state. An induction/neo-expression of HLA-D/Ii molecules has been determined in smooth muscle cells in human atherosclerotic plaque (Hansson et al. 1986) and in murine vasculitis (Moyer and Reinish 1984) as well as in malignant schwannomas and in the visceral nervous system in Crohn's disease and in chronic inflammatory demyelinating polyneuropathy (Pollard et al. 1986; Koretz et al. 1987). A microtopographic association between HLA-D/Ii-positive tumour cells and inflammatory stromal infiltrates, however, has in our series of STT only been observed in the case of a clear cell sarcoma of tendons and aponeuroses which, like malignant melanoma, is considered to be of neuroectodermal origin (Enzinger and Weiss 1988). The optional expression of MHC class II/Ii antigens in malignant fibrous histiocytomas might be explained by the possible monocyte/macrophage origin of this tumour type. In contrast, expression of these antigens in neoplastic cells of some osteosarcomas observed in this study has also to be considered aberrant since in situ normal osteoblasts lack any detectable HLA-D/Ii determinants.

Since this study indicated no induction/neo-expression of class I and class II/Ii molecules in STT in microtopographic association with an inflammatory infiltrate, it is proposed that alterations in MHC class I expression in STT be caused by factors other than local cytokine effects.

Concluding remarks

The ever-growing number of well-characterized cell surface molecules has opened a new dimension in the understanding of the biology and in the differential diagnosis of neoplasia. Distinct pathways and stages of cellular differentiation have been found to be associated with specific patterns of cell surface antigen expression. Thus, the assessment of cell surface phenotypic changes plays a central role in the classification of haematopoietic malignancies, malignant melanomas and even neuroblastomas (Rettig et al. 1987; Lennert and Feller 1990). In contrast to haematopoietic or neuroectodermal tissues, little is known about surface antigenic phenotypes of mesenchymal cells and their tumours.

This study represents a first attempt at phenotyping STT by means of cell surface molecules.

Selection criteria for the antigens examined

The study focused on the distribution pattern of CD antigens. These molecules are well-characterized, at least biochemically, and monoclonal antibodies carrying a CD designation have been extensively tested and characterized at international workshops. In addition, the rap-

Table 6. Frequency of cell surface molecule expression in neoplastic cells of STT

Phenotype	n	CD53	CD34	CD36	CD10	CD13	CD56	CD57	CD24	HLA-A,B,C/ β_2m	HLA-D/Ii
Leiomyoma	4	–	–	–	4	2	3	–	1	4	1
Leiomyoblastoma	1	–	–	–	–	1	1	–	–	1	–
Leiomyosarcoma	16	–	1	2	13	8	10	–	3	14	1
Rhabdomyosarcoma	8	–	2	2	3	–	8	–	–	6	–
Fibroma	2	–	–	–	–	–	2	–	–	–	–
Aggressive fibromatosis	3	–	–	–	3	3	3	–	–	3	–
Fibrosarcoma	4	–	–	–	2	2	3	–	–	2	–
Synovial sarcoma	5	–	–	–	3	4	3	–	1	3	–
Malignant fibrous histiocytoma	12	4	–	2	2	9	5	–	1	12	4
Chondrosarcoma	3	–	–	–	–	–	2	–	1	3	–
Osteosarcoma	4	2	–	–	4	4	4	–	4	4	–
Neurilemoma	3	–	–	–	1	–	3	1	–	3	–
Granular cell tumour	1	–	–	–	–	–	1	–	–	1	–
Malignant schwannoma	14	–	4	–	4	5	10	2	5	12	–
Peripheral neuroepithelioma	5	–	–	–	–	–	1	–	1	5	–
Ewing's sarcoma	1	–	–	–	1	1	–	–	–	1	–
Ganglioneuroma	4	–	–	–	–	–	4	4	–	4	4
Ganglio-neuroblastoma	5	–	–	–	–	–	5	2	5	2	–
Neuroblastoma	4	–	–	–	–	–	4	–	4	–	–
Lipoma	2	–	–	2	–	–	–	–	–	–	–
Liposarcoma	11	–	2	4	7	5	5	–	6	7	–
Lymphangioma	1	–	1	1	–	–	–	–	–	1	–
Haemangioma	4	–	4	4	–	–	–	–	–	4	–
Haemangio-pericytoma	6	–	1	2	–	–	2	–	–	6	1
Haemangiosarcoma	1	–	1	1	–	–	–	–	–	1	–
Epithelioid sarcoma	1	–	1	–	–	1	1	–	–	1	–
Alveolar soft part sarcoma	1	–	–	–	–	–	–	–	–	1	–
Clear cell sarcoma	2	–	–	–	–	–	–	–	–	2	1

idly growing understanding of the molecular structure of CD antigens indicates that a considerable number of these determinants are identical to cell adhesion molecules, enzymes, cytokine or even growth factor receptors (Knapp et al. 1989). Therefore, CD antigens might be of help in the understanding of cell-cell or cell-matrix interactions and to elucidate the biological behaviour of tumours. This may also be true for STT, since there is increasing evidence for the expression of CD antigens in non-neoplastic mesenchymal cells and in their tumours. Furthermore, given the observed alterations in MHC class I and class II/Ii antigens in the course of malignant transformation of epithelial, haematopoietic and neuroectodermal cells (Ruiter et al. 1984; Möller et al. 1987; Koretz et al. 1989), these molecules might be a vehicle in the prediction of the malignant potential of STT.

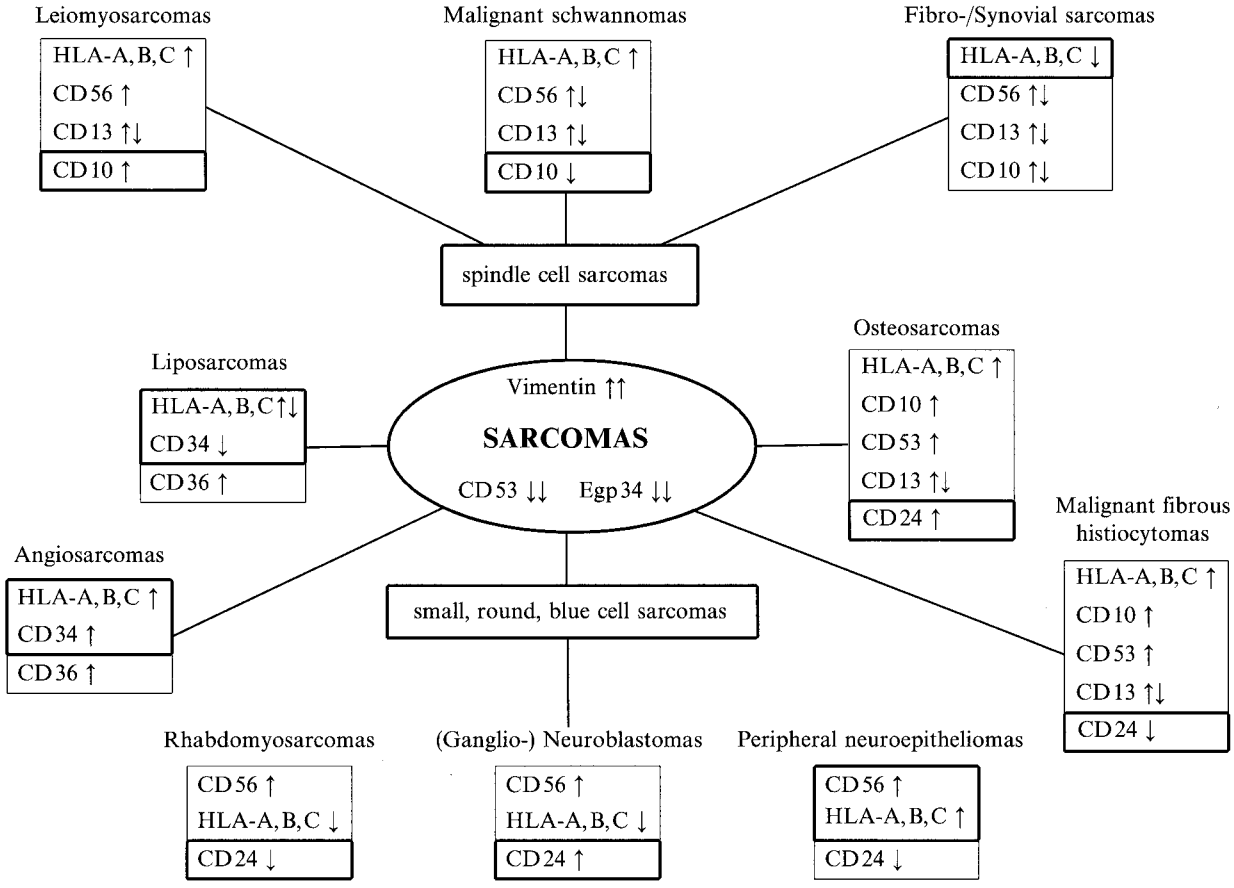
The stromal component of STT

In contrast with carcinomas, where a basal lamina often separates clumps of malignant cells from islets of tumour stroma, individual tumour cells in STT about directly on and may intermingle with stromal elements. This renders the differentiation between neoplastic cells and reactive stromal cells most difficult. Moreover, a considerable number of CD antigens as well as MHC class I and class II/Ii antigens are also expressed by lympho-histiocytic stromal infiltrates, a feature which must be taken into consideration when assessing the immunoprofile of neoplastic cells in STT. Therefore, a distinct and reliable visualization of the lympho-histiocytic stromal infiltrate is mandatory to allow its clear discrimination from neoplastic cells. In this study, this problem was overcome by incubation of one of the serial sections of each case with CD53 mAb HD77. CD53 antigen not only allowed the visualization of the entire lympho-histiocytic stromal infiltrate of the tumours, it also revealed STT to generally appear devoid of significant inflammatory infiltrates.

Overall expression of the antigens examined in STT

Table 6 summarizes the distribution frequency of all antigens examined in STT irrespective of the amount of

Table 7. Useful immunophenotype configurations in differential diagnosis of STT



Arrows indicate antigen expression. ↑, Frequent; ↓, rare; ↑↓, varying. Framed boxes (*thick lines*) indicate antigens that discriminate between tumour types juxtaposed. Framed boxes (*thin lines*) show antigenic profiles that are frequently found in the corresponding tumour types

neoplastic cells expressing the reactive antigens. HLA-A, B,C/β_2m and CD56 antigens were those most broadly distributed in STT and often showed an overlapping pattern of expression. They also did exhibit a complementary distribution pattern in (ganglio-)neuroblastomas and in peripheral neuroepitheliomas. HLA-A,B,C/β_2m molecules, in particular, displayed considerable changes in the expression pattern in STT compared to their non-neoplastic counterparts which mainly consisted in an aberrant induction/neo-expression of these molecules. CD24, although less frequently found in STT than was CD56, shared with the latter the consistent expression in all (ganglio-)neuroblastomas studied. CD10 and CD13, which both belong to the metalloprotease family, were also frequently observed in STT. Their binding pattern was overlapping in a considerable number of cases but not in all STT. Remarkably, the great majority of malignant fibrous histiocytomas and leiomyosarcomas expressed CD10 molecules. CD53, CD34, CD36 and CD57 antigens were only rarely found in STT. CD53 was restricted to some tumours of likely monocyte/macrophage origin. CD57, which was extremely scarce in STT, was solely restricted to tumours of neural tissue. CD34 and CD36 exhibited a comparable expression in vascular tissues, the latter being also consistently expressed in well-differentiated tumours of adipose tissue.

Impact of cell surface molecules in the differential diagnosis of STT

Based on the current classifications of STT, the cell surface immunophenotypes were studied with respect to their possible value in the differential diagnosis of STT (illustrated in Table 7). The following immunophenotypic findings are suggested to be of value in the differential diagnosis of STT:

1. Egp34, which was only found in a tumour cell subpopulation of 1/128 STT, is a very useful tool to discriminate between STT and epithelial neoplasias.
2. Except for some malignant fibrous histiocytomas and osteosarcomas, which are considered to be histogenetically related to the monocyte/macrophage system, the STT studied were devoid of any detectable CD53 molecules. Thus, despite the broad distribution pattern of CD antigens in STT, CD53 is absent in nearly all STT and allows their discrimination from haematopoietic malignancies.

3. CD53 expression in neoplastic cells of STT was an exclusive characteristic of malignant fibrous histiocytomas and osteosarcomas. Both tumour types, in addition, showed expression of HLA-A,B,C/β_2m and CD10 molecules in all cases, although not always consistently.
4. The co-expression of CD10, CD13, CD56 and HLA-A,B,C/β_2m molecules was a common feature in aggressive fibromatoses whereas the combined expression of these molecules was rarely observed in fibromas or in their malignant counterparts.
5. Among the spindle cell sarcomas which comprised malignant schwannomas, leiomyosarcomas and fibrosarcomas/monophasic fibrous synovial sarcomas and which revealed a high occurrence of CD56 expression, fibrosarcomas/monophasic fibrous synovial sarcomas showed a tendency towards partial or even complete absence of HLA-A,B,C/β_2m molecules, while they were regularly expressed in the vast majority of malignant schwannomas and leiomyosarcomas.
6. CD10 expression which was frequently determined in leiomyosarcomas but only rarely in malignant schwannomas might be, at least in combination with other immunophentoypic characteristics, an argument in favour of leiomyosarcoma.
7. CD34 and CD36 were found to be reliable markers for vascular endothelial cells and their neoplastic counterparts. In addition, consistent expression of CD36 was found in well-differentiated tumours of adipose tissue.
8. The most discriminating results were obtained for small, round, blue cell sarcomas: the presence of HLA-A,B,C/β_2m by simultaneous absence of CD56 discriminated peripheral neuroepitheliomas from (ganglio-)neuroblastomas and rhabdomyosarcomas which, in contrast, were all CD56-positive. Peripheral neuroepitheliomas also contrasted with (ganglio-)neuroblastomas by the frequent absence of CD24 which was identified in all (ganglio-)neuroblastomas. (Ganglio-)neuroblastomas and rhabdomyosarcomas which shared CD56 expression while lacking HLA-A,B,C/β_2m molecules in the majority of cases, might be discriminated from each other by CD24 expression which was restricted to (ganglio-)neuroblastomas.

State-of-the-art and prospects

Some immunophenotype configurations observed in the comprehensive series of STT studied here fit into the current classification systems. They are therefore suggested as a useful supplement to the differential diagnosis of this complex tumour entity. One should bear in mind, however, that the absolute number of different types and subtypes of STT studied is still relatively small. It is thus hoped that the present study provides a stimulus to examine additional cases of STT to augment the number of STT investigated for cell surface expression. In some STT, the cell surface antigenic profile corresponded to that of their non-neoplastic counterparts although the majority of STT showed an aberrant induction/neo-expression or, to a much lesser extent, an aberrant abrogation/loss of these molecules. Accordingly, the cell surface immunophenotype of STT might at some point aid with the prediction of the biological behaviour of STT. Finally, the detailed cell surface immunophenotyping of STT might be a first step towards the use of CD mAb for diagnostic imaging and for new treatment regimens introducing the clinical application of monoclonal antibodies or biological response modifiers.

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