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ORIGINAL ARTICLE

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Identification of CD56 and CD57 by flow cytometry in Ewing's sarcoma or primitive neuroectodermal tumor

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Abstract CD56 and CD57 are commonly considered as natural killer and neuroectodermal markers, but their expression has been identified in a wide spectrum of neoplasms including some cases of Ewing's sarcoma (ES) and primitive neuroectodermal tumor (PNET). We report two cases of small, round blue cell tumor (SRBCT), in which flow cytometry immunophenotyping (FCI) detected strong expression of CD56 and CD57 (one case). Immunohistochemical staining with Leu-19 and Leu-7 confirmed the FI results. Although CD56 and CD57 expression is consistent with ES/PNET, it can be potentially misleading if results of FCI are interpreted in the absence of other findings. These cases suggest the utility of FCI in undifferentiated SRBCT. The literature on CD56 and CD57 expression in ES/PNET is reviewed and discussed.

Key words Ewing's sarcoma · Primitive neuroectodermal tumor · Flow cytometry · Immunophenotyping · NCAM · Antigens

Introduction

CD56, one of the isoforms of neural cell adhesion molecule (NCAM), is an integral membrane protein, which has been identified as a natural killer cell antigen [9]. In addition, it has been identified in neuroendocrine neoplasms and some soft tissue and bone tumors, including undifferentiated small, round blue cell tumors (SRBCT) [4, 5, 16]. The related antigen CD57 has also been described on natural killer cells, neuroectodermal and neural tumors, and other tumors [1, 13, 15]. For these reasons, CD56 and CD57 have been used as immunohistochemical markers of neuroendocrine tumors and some SBRCT. Previous studies have used immunohistochemistry on frozen tissue [4, 5, 13, 16], formalin-fixed paraffin-embedded tissue [15], or flow cytometric immunophenotyping (FCI) of tumor cell cultures [10, 24]. To our knowledge, utilization of FCI for detection of these markers on tumor cell suspensions of clinical samples has not been described.

We describe two cases diagnosed as having tumors belonging to the Ewing's sarcoma (ES) and primitive neuroectodermal tumor (PNET) family, in which FCI revealed expression of CD56 and CD57 (one case). FCI was not only useful in identifying CD56 and CD57 expression, but also excluded the other diagnostic possibility considered in both cases – malignant lymphoma.

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Case reports

Case 1

The first case is that of a 13-year-old boy with a mediastinal and abdominal mass. On frozen section, this was diagnosed as an undifferentiated SRBCT, a portion of which was sent for FCI since malignant lymphoma was a diagnostic consideration. Pleural fluid was also submitted for flow cytometry. Wright-stained cytopsin preparations of both cell suspensions showed malignant cells of varying sizes with vacuolated bluish cytoplasm. Hematoxylin-eosin (HE)-stained tissue sections (Fig. 1) revealed an undifferentiated round cell tumor invading fibrovascular and adipose tissue, with tumor necrosis and a brisk mitotic rate. Homer-Wright rosettes were not identified. A PAS stain with and without diastase showed the presence of glycogen in tumor cells. Tumor cells were also diffusely positive for O-13 (CD99) (DAKO, Carpinteria, Calif.). LCA (CD45), S-100, synaptophysin, pan-cytokeratin, and desmin (DAKO) were negative. Vimentin (DAKO) was weakly positive. In addition, Leu-19 (CD56) and Leu-7 (CD-57) (DAKO) immunostains were subsequently performed and were strongly positive. Electron microscopy showed cells with intermediate filaments, microtubules, a few membrane-bound dense granules, vacuoles and lipid droplets and poorly developed cellular junctions. There was no evidence of muscle differentiation. A diagnosis of

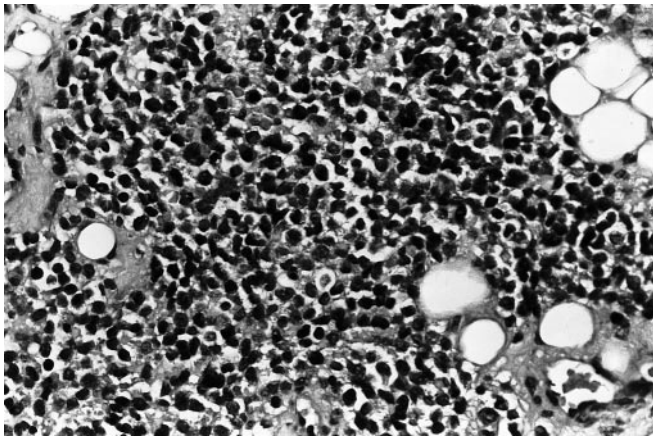

Fig. 1 Tissue section from case 1. HE, ×400

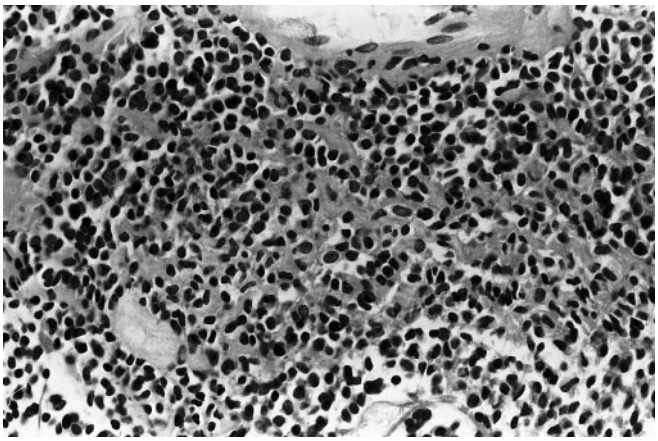

Fig. 2 Tissue section from case 2. HE, ×400

ES/PNET was made. The patient received two cycles of chemotherapy with cyclophosphamide, ifosphamide, and VP-16 as per PNET protocol. A restaging MRI showed a major response of the abdominal disease. He received two more cycles of chemotherapy with ifosphamide, VP-16, cyclosporin, and Adriamycin. A repeat CT scan showed no evidence of tumor, and the boy received a bone marrow transplant, which was complicated by infections. Five months later, the patient relapsed with a rectal mass, porta hepatis mass and peritoneal studding. In the 6 months since this relapse, the patient has undergone several cycles of chemotherapy, with alternating improvement and then relapses of the tumor.

Case 2

The second case is that of a 44-year-old man, who presented with a large mass involving the bone of the iliac crest and adjacent soft tissue. Clinically, this was felt to be a primary bone neoplasm. Both bone marrow biopsy and biopsy of the soft tissue mass were performed. Tissue was submitted for FCI, since malignant lymphoma was a diagnostic consideration. Wright-stained touch preparations of the tissue showed small to intermediate-sized cells with smudged nuclear chromatin and small amounts of cytoplasm. HE-stained tissue sections showed fibrous tissue infiltrated by strands, nests, and sheets of small to intermediate-sized cells with dark nuclei and scant cytoplasm (Fig. 2). Homer-Wright rosettes were not identified. Immunoperoxidase stains performed on paraffin-embedded tissue showed intense positivity for O-13 and neuron-specific enolase (DAKO). Smooth muscle actin, desmin (DAKO), S-100 (Biogenex, San Ramon, Calif.), chromogranin (DAKO), synaptophysin, low-molecular-weight cytokeratin (DAKO) and LCA were all negative. Leu-19 immunostain was positive, but, the Leu-7 immunostain was negative. A diagnosis of ES/PNET was made. The patient started chemotherapy and died of complications 2 weeks after diagnosis. An autopsy was not performed.

Material and methods

The specimen in case 1 consisted of mediastinal biopsy and pleural fluid. Core needle biopsy of iliac mass was received in case 2. Fresh tissue obtained for FCI was transported in RPMI 1640 cell medium (Cellgro Mediatech tissue Culture Media; Fisher Scientific, Pittsburgh, Pa.). Single cell suspensions were prepared from fresh tissue. The suspensions were then analyzed on Ortho Cytoronabsolute (Ortho Diagnostic Systems, Raritan, N.J.) and Facsan (Becton Dickinson Immunocytometry Systems, Mountain View, Calif.) flow cytometers in cases 1 and 2, respectively, for various antigens, using standard techniques and the commercially-available monoclonal antibodies (Mab) listed in Table 1.

Results

The results of FCI are shown in Table 2. In summary, in both cases, tumor cells were present mainly in the monocyte region and mixed with T lymphocytes in the lymphocyte region. The cells uniformly expressed CD56 and CD57 in case 1 and CD56 in case 2. They lacked expression of CD45, CD16 (case 1), and CD2. The pattern of

Table 1 Commercial sources of monoclonal antibodies

Monoclonal antibody	Commercial source
CD1	Ortho Diagnostic Systems, Raritan, N.J.
CD2	Coulter Immunology, Hialeah, Fla.
CD3	Becton-Dickinson, San Jose, Calif.
CD4	Becton-Dickinson
CD5	Gen Trak Inc., Wayne, Pa.
CD7	Becton-Dickinson
CD8	Gen Trak
CD10	Coulter Immunology
CD11c	Dako, Carpinteria, Calif.
CD13	Coulter Immunology
CD14	Coulter Immunology
CD15	Ortho Diagnostic Systems
CD16	Ortho Diagnostic Systems
CD19	Coulter Immunology
CD20	Coulter Immunology
CD24	Coulter Immunology
CD33	Coulter Immunology
CD34	Coulter Immunology
CD38	Ortho Diagnostic Systems
CD45	Becton-Dickinson
CD56 (N901)	Coulter Immunology
CD57 (Leu-7)	Becton-Dickinson
CD117	Coulter Immunology
BB4	Biotest Diagnostics, Denville, N.J.
IgG	Ortho Diagnostic Systems
IgM	Ortho Diagnostic Systems
IgA	Ortho Diagnostic Systems
IgD	Ortho Diagnostic Systems
Kappa	Ortho Diagnostic Systems
Lambda	Ortho Diagnostic Systems
TdT	Coulter Immunology
HLA-DR	Ortho Diagnostic Systems

Table 2 Results of flow cytometry immunophenotyping in cases 1 and 2 (*ND* not done)

Mab or cells in regions	Case 1, mediastinal tissue		Case 1, pleural fluid		Case 2, iliac mass tissue	
	Lymphocyte region	Monocyte region	Lymphocyte region	Monocyte region	Lymphocyte region	Monocyte region
Cells in the region	25%	40%	2%	92%	6%	24%
CD1	ND	ND	4%	1%	ND	ND
CD2	ND	ND	68%	2%	34%	2%
CD3	3%	2%	63%	0%	29%	1%
CD4	2%	1%	35%	3%	16%	2%
CD5	2%	1%	64%	1%	34%	1%
CD7	ND	ND	70%	2%	ND	ND
CD8	1%	1%	23%	0%	25%	22%
CD10	54%	30%	4%	2%	3%	3%
CD11c	33%	23%	ND	ND	ND	ND
CD13	ND	ND	6%	10%	ND	ND
CD14	4%	4%	0%	3%	1%	1%
CD15	ND	ND	4%	2%	ND	ND
CD16	1%	0%	7%	2%	ND	ND
CD19	1%	0%	4%	0%	4%	0%
CD20	80%	42%	9%	0%	0%	1%
CD24	ND	ND	3%	0%	ND	ND
CD33	ND	ND	1%	4%	ND	ND
CD34	ND	ND	3%	4%	ND	ND
CD38	ND	ND	44%	3%	0%	1%
CD45	8%	4%	82%	2%	29%	8%
CD56	78%	95%	51%	99%	62%	95%
CD57	76%	92%	28%	93%	ND	ND
CD117	ND	ND	1%	5%	ND	ND
BB4	ND	ND	9%	9%	17%	14%
IgG	ND	ND	1%	3%	10%	31%
IgM	ND	ND	2%	1%	24%	23%
IgA	ND	ND	2%	0%	10%	16%
IgD	ND	ND	3%	0%	6%	6%
Kappa	1%	4%	3%	3%	5%	14%
Lambda	2%	5%	3%	3%	8%	16%
TdT	0%	ND	0%	ND	ND	ND
HLA-DR	15%	11%	11%	5%	19%	14%

immunofluorescence showd strong expression of both CD56 and CD57 (Fig. 3).

Discussion

ES is an uncommon neoplasm of bone and extraosseous tissue, commonly presenting as an SRBCT. In morphological and clinical features it is similar to PNET. Occasional expression of neuroectodermal markers, such as neuron-specific enolase (NSE), neurofilament [25], nerve growth factor receptor [10] and Leu-7 (CD57) [15, 19] suggests a relationship of ES to neuroectodermal tumors. ES also shares with PNET specific chromosomal transformations, with t (11; 22) being most common, and expression of CD99 (p30/32 product of MIC2 gene), as detected by immunostaining with Mab O13 or HBA-71 [8]. Thus, differentiation of ES from PNET is difficult, but it can be important because of reported worse patient survival in PNET than in ES. Recent studies comparing ES and PNET for survival have relied on expression of at least two neuroectodermal markers for distinction of PNET from ES cases [2, 22].

NCAM is a member of the cell adhesion molecules family and plays a part in development, regeneration, and possibly oncogenesis. Multiple isoforms of NCAM have been isolated. These result from alternative splicing and polyadenylation of mRNA transcribed from a single gene located on chromosome 11. Additional diversity of NCAM is produced by distinct patterns of post-translational glycosylation. NCAM expression is abundant during early development but restricted mostly to neural cells in adults [13]. However, antigen cluster CD56 has been shown to be a 140-kDa isoform of NCAM [9] expressed by natural killer cells of lymphoma and related lymphoproliferative disorders. In addition, NCAM was detected by an immunohistochemical study in adrenal gland, reactive skeletal muscle fibers, cardiac muscle, visceral smooth muscle, ovarian and prostatic tissue cervical stroma, thyroid epithelium, gastric epithelium, intestinal lamina propria, Leydig cells of testis, and islet cells of the pancreas [5]. The same study also documented expression or neoexpression of NCAM by a variety of neoplasms, including small cell carcinoma, other neuroendocrine tumors, neuroblastoma, medulloblastoma, schwannoma, glioma, meningioma, rhabdomyosarcoma,

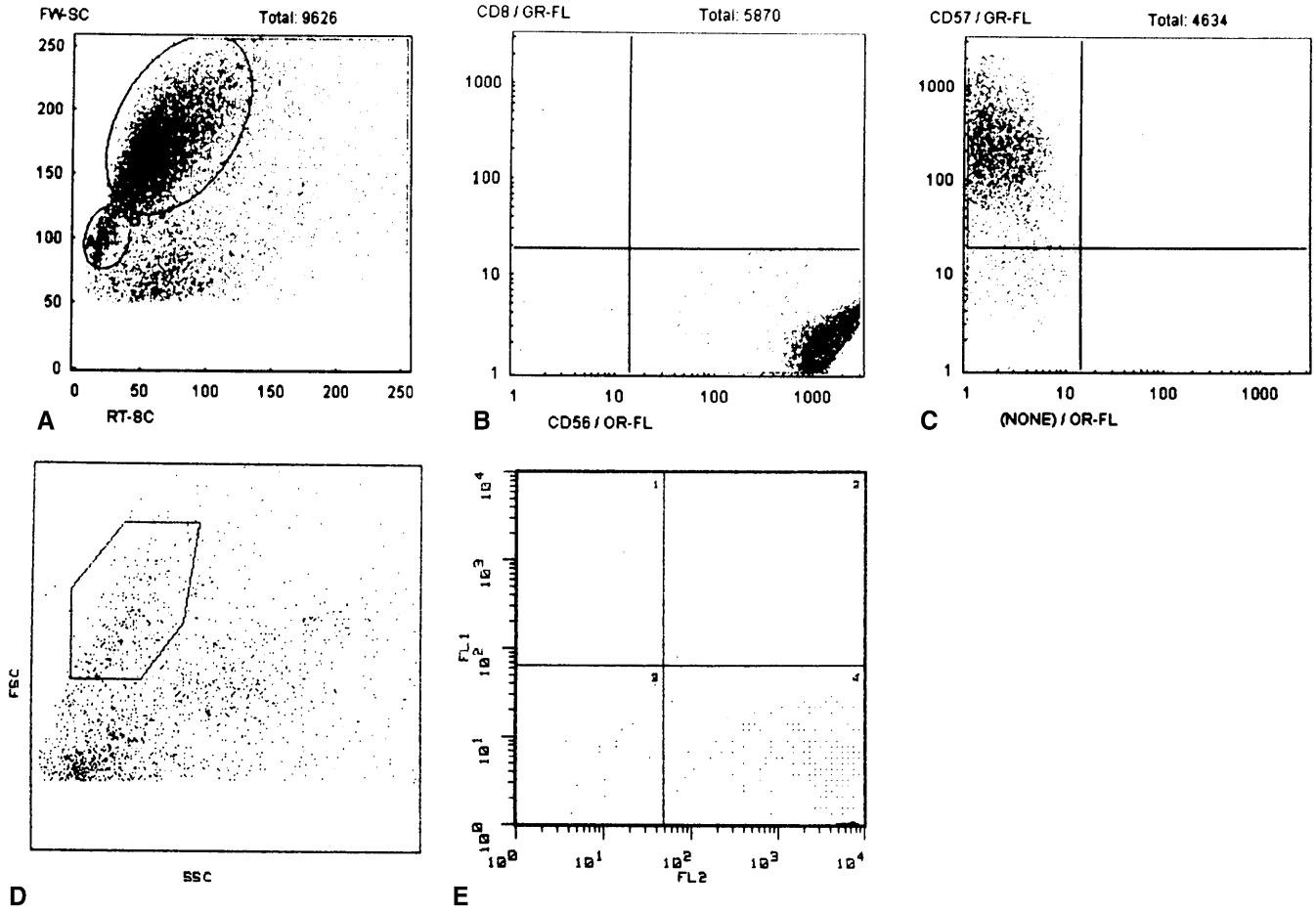

Fig. 3 FCI of tumor cell suspensions. **A–C** in case 1 and **D, E** in case 2. **A** Forward and right-angle light scatters. **B** CD56 vs CD8, monocyte region. **C** CD57 only, monocyte region. **D** Forward and right-angle light scatters, monocyte region *marked.* **E** CD56 only, monocyte region

uterine leiomyoma, synovial sarcoma, chondrosarcoma, some osteosarcomas, and even a few large cell carcinomas, mainly of breast and renal origin. Other studies confirmed these results and also showed expression of NCAM in Wilms' tumor [17], mixed müllerian tumor, malignant fibrous histiocytoma, hemangiopericytoma [16], some melanomas [4], and hematopoietic malignancies, such as multiple myeloma and a subset of myeloid leukemia [6].

Expression of NCAM by PNET has been demonstrated in minority of cases (2 of 11, 1 of 5, and 2 of 6) by immunohistochemical studies [5, 12, 14]. Expression of NCAM by ES appears controversial. Seemingly, conflicting data have been reported. Early studies with rabbit antiserum against purified mouse NCAM, rat antibody P61 against mouse NCAM, and UJ13A, and anti-NCAM Mab raised against human fetal brain demonstrated NCAM in most ES cell lines by FCI [19]. Similar results were obtained with indirect immunofluorescence with Mab UJ13A and rabbit anti-mouse NCAM antiserum on ES lines [17]. Analysis of NCAM in ES cell lines showed the presence of 140-, 120-, and 180-kDa slow-sialylated NCAM, but it was considerably less abundant than in neuroblastoma cell lines [11]. Recently, the presence of 140-kDa NCAM on ES cell lines was confirmed by FCI with 6H7 and Leu-19 antibodies [24]. A study with Mab 3F8, raised following immunization of mice with neuroblastoma cells and recognizing sialic acid residues epitope of NCAM, showed indirect immunofluorescence reactivity of all four studied cell lines of ES [18]. On the other hand, immunohistochemical studies have generally failed to show NCAM expression in ES tissue. Such studies used Mab Leu-19 [12, 14] or Mab 5.H11, raised against human myoblasts in culture and reactive with different isoforms of NCAM [5]. The last study cited identified only one 5.H11-positive case among 20 ES studied.

The complexity of NCAM expression in ES is further compounded by the results of ES testing with CD56 cluster antibodies. Two of nine cases of ES reacted with Mab N901, but none reacted with T-199 or Leu-19, other CD56 antibodies tested [3]. This study supports the premise that the conflicting reports on NCAM expression in ES can be partially explained by the use of Mab reacting with different epitopes and isoforms of NCAM. The NCAM epitope recognized by N901 (NKH1b) is different from epitopes recognized by Leu-19 (NKH1a) or T-199 (NKH1c) [23].

Various study techniques used by different researchers can also account for some discrepancies. As the above reports show, studies using ES cell lines and FCI or immunofluorescence were more likely to show NCAM expression than immunohistochemical studies of ES tissue sections. That can be due to NCAM expression induced by cell culture environment or cell lines unrepresentative of the majority of ES, or simply reflect different sensitivities of NCAM detection by different methods. In addition, confusion of ES with PNET cases cannot be excluded, given the extensive similarities of the tumors.

CD57 (HNK-1) is an epitope present in some NCAM types [7]. It can be detected in tissue with the use of Leu-7 Mab, initially described as a marker of natural killer cells [1]. Its expression has also been detected in schwannoma, ganglioneuroma, neuroblastoma, neuroendocrine carcinoma, Askin tumor, rhabdomyosarcoma, 2 of 3 skeletal ES, and 3 of 6 PNET cases [13, 15]. Another immunohistochemical study showed Leu-7 expression in 12 of 40 ES of bone [19].

The present report presents two cases diagnosed as ES/PNET with CD56 expression detected with N901 Mab by routine diagnostic FCI and confirmed by immunohistochemistry with Mab Leu-19. In addition, expression of CD57 was detected in case 1 with the use of FCI and immunohistochemical stain with Mab Leu-7. These findings support the presence of CD56 and CD57 expression in selected cases of ES or PNET.

Our study suggests the utility of FCI as means of routine diagnostic immunophenotyping of ES/PNET, similar to the use of FCI for ES cell lines immunophenotyping in research [10, 24]. Although the expression of CD56 is unlikely to have diagnostic significance in ES or PNET, FCI can be adapted for detection of other diagnostic markers, such as CD99. However, it should be emphasized that CD99 expression must be interpreted together with hematopoietic markers, because of common CD99 expression in lymphoblastic lymphomas and leukemias [21]. FCI may allow for reliable exclusion of the diagnosis of malignant lymphoma or leukemia, since lymphoma or leukemia is frequently considered in the differential diagnosis of a small, round blue cell tumor. Lymphoblastic malignancies can be CD45 negative by paraffin immunohistochemistry [20] and can require extensive frozen section immunophenotyping or FCI for proper diagnosis.

Finally, this is the first report showing a possibility of a pitfall in the diagnosis of SRBCT when FCI detects expression of CD56 and/or CD57, commonly known as natural killer cell-asssociated markers, and suggests a natural killer cell phenotype. However, additional markers commonly present in natural killer cells (CD2, CD16, and CD45) can be studied by FCI to exclude this possibility.

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