Histiocytic sarcoma: a study of five cases including the histiocyte marker CD163

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Histiocytic sarcoma (HS) is a rare but controversial hematopoietic neoplasm. In the past, malignancies have been misclassified as histiocytic tumors due to overlapping histologic features and inadequate phenotypic data. CD163, a recently characterized hemoglobin scavenger receptor, appears to be a 'specific' marker of histiocytic lineage and a promising diagnostic tool for evaluating histiocytic neoplasms. Five cases of HS were studied to further elucidate the clinicopathologic features of these rare tumors and to demonstrate the diagnostic utility of CD163. Criteria for diagnosis included histologic and immunohistochemical evidence of histiocytic differentiation, CD45 positivity, and exclusion of lymphoid, epithelial, melanocytic and dendritic cell phenotype. Sites of disease included the colon (two cases), palate, inguinal lymph node, and testis. The clinical course was aggressive in 4/5 patients (survival = 2–15 months). One patient with localized disease of the palate, survived 17 years after diagnosis. All patients with poor survival had tumors ≥ 3.5 cm. Histologically, all cases showed diffuse architecture with large, discohesive polygonal cells. Spindling of cells was focally noted. Hemophagocytosis was identified in 3/5 cases. A prominent inflammatory background was present in 4/5 tumors. All cases were immunoreactive for CD45, CD163, CD68, and lysozyme. S-100 was focally positive in 4/5 cases. Antibodies for melanocytic, epithelial, lymphoid, and dendritic cell markers were negative. Molecular studies showed monoclonal IgH gene rearrangements in three cases. Our findings suggest that HS is an uncommon neoplasm frequently extranodal in presentation and aggressive in behavior, with rare exceptions. Stage of disease and possibly tumor size are significant prognostic indicators. Molecular studies remain controversial in the diagnosis. The morphologic and phenotypic features are relatively uniform; however, the diagnosis requires exclusion of more common neoplasms by extensive immunophenotypic studies. CD163 appears to be a specific histiocytic marker and is important in establishing the diagnosis of HS.

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Histiocytic sarcoma (HS), formerly designated as true histiocytic lymphoma, consists of exceedingly rare hematopoietic neoplasms, representing <1% of all non-Hodgkin's lymphomas.^{1,2} Many inconsistencies in the terminology and diagnostic criteria of these lesions have historically complicated their recognition and characterization. In fact, neoplasms originally classified as 'reticulum cell sarcomas'³ and later 'histiocytic lymphomas' by Rappaport⁴ have encompassed a biologically heterogeneous

group of disorders, the majority of which are now known to be high-grade T- or B-cell, non-Hodgkin lymphomas.^{5–10}

The term 'histiocytic sarcoma' was introduced in 1970 by Mathé *et al*.¹¹ however, this and previous descriptions were based strictly on the histologic similarities of the cells to macrophages. Since these original papers, increasing emphasis has been placed on cytochemical and immunohistochemical characterization of these neoplasms in an attempt to establish true histiocytic lineage.^{12–17} This diagnostic challenge is further complicated as it is necessary to distinguish HS from other histiocytic processes, both benign and malignant, to include the hemophagocytic syndrome,^{18–20} malignant histiocytosis,^{10,18–21} and monocytic leukemia.^{17,19}

The diagnosis of HS relies predominantly on the verification of histiocytic lineage and the exclusion of other, poorly differentiated, large cell

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malignancies (ie lymphoma, carcinoma, melanoma) by way of extensive immunophenotypic investigation. Fulfilling these conditions, particularly establishing histiocytic differentiation, has been problematic due to a high incidence of cross-reactivity of many immunohistochemical antibodies originally believed to be specific for histiocytes.^{22–24} The recent characterization of CD163,^{25,26} a hemoglobin scavenger receptor protein, has offered a means of identifying histiocytic cells with a greater degree of specificity and is a promising marker in the diagnosis of true histiocytic malignancies. Finally, the contribution of molecular gene rearrangement studies as a diagnostic tool for histiocytic tumors remains unclear.^{14,27–29}

At the present time, the World Health Organization defines HS as a malignancy with morphologic and immunophenotypic features that resemble those of mature tissue histiocytes.² Despite the application of more stringent diagnostic criteria; however, an increasing number of reports continue to shed light on these uncommon malignancies.^{9,30–44} Extranodal presentation is frequent and the clinical course is generally aggressive. Unfortunately, persistent clinical and histopathologic disparities in these studies, in addition to the overall rarity of these neoplasms, have continued to obscure this diagnosis and prevented a full appreciation of their clinical behavior. We report the clinicopathologic features of five cases of HS to further elucidate the intriguing nature of these rare malignancies and introduce the application of a novel immunohistochemical marker, CD163, to attempt to verify the exact lineage of these neoplasms.

Materials and methods

Specimens and Selection Criteria

Five cases of HS were selected from 19 that were originally diagnosed as ‘histiocytic sarcoma,’ ‘true histiocytic lymphoma’ or ‘interdigitating dendritic cell/histiocytic sarcoma’ from the files of the Armed Forces Institute of Pathology between 1985 and 2001. Most of the excluded cases were reclassified following immunohistochemical analysis. Three cases were excluded at the onset of the study because they lacked paraffin blocks for ancillary studies. Case inclusion required morphologic and immunophenotypic features consistent with histiocytic lineage (CD68, CD163, lysozyme, MAC387 reactivity) and the absence of immunoreactivity for an extensive panel of lymphoid, accessory/dendritic cell, epithelial, and melanocytic markers. Cases not meeting these criteria, and those that lacked CD45 (LCA) expression, were excluded. In addition, patients with a leukemic or widely disseminated initial presentation were not considered in the present study.

Clinical History

Medical records were retrieved for all cases in this study. Available records were reviewed for clinical presentation, tumor size, stage, treatment, and clinical outcome.

Histology and Immunohistochemistry

Hematoxylin–eosin slides were reviewed in all cases. An extensive panel of immunohistochemical antibodies was applied to 4 μ m, formalin-fixed, paraffin-embedded tissue sections according to standard methods. A list of the primary antibodies, clones, vendors, pretreatment regimens, and working dilutions used in this study are provided in Table 1. The sections were deparaffinized and treated with the specified antigen retrieval method, followed by overnight incubation with the primary antibodies at room temperature. Endogenous enzyme activity was quenched by treating the tissue with 3% hydrogen peroxide for 10 min. A streptavidin–biotin–peroxidase system (Vectastain Elite[®] ABC Kit, Vector Laboratories, Burlingame, CA, USA) with a diaminobenzidine (DAB) chromogen was then used to develop the stain. Sections were counterstained with Gill’s hematoxylin, dehydrated in ethanol and cleared in xylene. Appropriate positive and negative controls were used for all antibodies.

Immunohistochemical stains were interpreted as positive if at least 10% of the neoplastic cells showed intense immunoreactivity. Specimens were considered focally positive if immunoreactivity was identified within the cells of interest but comprised less than 10% of the tumor. Cell proliferation was examined by Ki-67 antibody staining.

Gene Rearrangement Studies

Molecular diagnostic studies were performed by polymerase chain reaction (PCR) for the immunoglobulin heavy chain (IgH), T-cell receptor β (TCR β) and T-cell receptor γ (TCR γ) genes, as previously described.^{45–48} DNA was extracted from formalin-fixed, paraffin-embedded tissues in all cases and purified according to previously published methods.^{46,48} Consensus primers were used for detection of IgH chain (FR3A V region and CFW1 J region), TCR β chain (VJ1, VJ2, D1J2, and D2J2), and TCR γ chain (V2, V3, V4, V8, V9, JGT3, JGT4, and JGT12) gene rearrangements. PCR analyses were performed using a Perkin–Elmer Thermal Cycler 9600 (Applied Biosystems, Foster City, CA, USA) for 40 cycles using the following parameters: initial 10 min incubation at 94°C, denaturation for 1 min at 94°C, annealing for 1 min at 52°C, elongation for 1 min at 72°C, and a final elongation step for an additional 5 min at 72°C. All reactions were performed in

Table 1 Immunohistochemical antibody panel

<i>Antibody</i>	<i>Clone^a</i>	<i>Source^b</i>	<i>Antigen retrieval</i>	<i>Dilution</i>
CD45RB	PD7/26, 2B11	Dako	Steam ^c	1:500
CD3	Polyclonal	Cell Marque	Steam	1:1000
CD5	4C7	Novocastra	Steam	1:1000
CD20	L26	Dako	Steam	1:800
CD79a	HM57	Dako	Steam	1:500
PAX5	24	Cell Marque	Steam	1:5
CD163	10D6	Novocastra	Steam	1:100
CD68	KP-1	Dako	Steam	1:25000
Lysozyme	Polyclonal	Dako	Steam	1:2500
MAC387	MAC387	Dako	Steam	1:500
Factor XIIIa	FXIIIa-subunit	Calbiochem	Protease1	1:1600
Fascin	55 K-2	Cell Marque	Steam	1:20
S-100	Polyclonal	Sigma	Steam	1:2000
HMB	HMB-45	Enzo Biochem	Steam	1:2000
Tyrosinase	T311	Novocastra	Trilogy (Cell Marque)	1:20
Melan A	A103	Novocastra	Trilogy (Cell Marque)	1:25
CD21	1F8	Dako	Pepsin	1:100
CD35	Ber-Mac-Drc	Dako	Pepsin	1:100
CD1a	O10	Immunotech	Steam	1:50
CD30	Ki-1, Ber-H2	Dako	Steam	1:300
CD34	Q bend	Immunotech	Steam	1:100
MPO	Polyclonal	Dako	Steam	1:1200
CD56	123C3	Caltag	Steam	1:100
Cytokeratin	AE1/AE3	Chemicon Intl	Steam	1:400
EMA	E29	Dako	Steam	1:200
LMP-1	CSI-4	Dako	Steam	1:500
Ki-67	MIB-1	Dako	Steam	1:500

^aAll antibodies are monoclonal unless otherwise indicated.

^bDako = Dako, Carpinteria, CA, USA; Cell Marque = Cell Marque, Hot Springs, AK, USA; Novocastra = Novocastra Lab Ltd, Newcastle upon Tyne, UK; Calbiochem, San Diego, CA, USA; Sigma = Sigma, St. Louis, MO, USA; Enzo Biochem = Enzo Biochem, Farmingdale, NY, USA; Immunotech = Immunotech, Miami, FL, USA; Caltag = Caltag, Burlingame, CA, USA; Chemicon Intl = Chemicon International, Temecula, CA, USA.

^cSteam antigen retrieval for 20 min in 1 mM EDTA.

duplicate along with positive and negative control specimens. The positive controls consisted of monoclonal cell lines (SUD 5, QA2#3, and Sup T-1 for IgH, TCR β and TCR γ , respectively) and the negative control specimens were benign tonsil tissue and sterile water. The products underwent separation and detection by capillary electrophoresis using an ABI Prism[®] 310 Genetic Analyzer (Applied Biosystems). The resultant banding patterns were analyzed for the presence of monoclonality.

Cases were reported as monoclonal if a dominant peak was observed on the electropherogram and was reproducible on duplicate tests (± 1 base pair) using the same specimen and primer set. A peak was considered dominant only when the amplitude was greater than all peaks present and was at least twice that of the local background. Reactions were determined to be negative (ie polyclonal) if multiple peaks were present but no dominant peak was detected or, in cases in which lymphocytes were sparse, a dominant peak was not reproducible on duplicate tests. Cases were considered not amplifiable (ie no PCR products were obtained) if the tracing was essentially the same as the water control.

Electron Microscopy

Representative biopsy samples from three cases were analyzed by electron microscopy. One case was extracted from the paraffin tissue block, deparaffinized with xylene and postfixed in glutaraldehyde. The remaining two cases were primarily fixed in glutaraldehyde and processed by conventional methods.⁴⁹ Sections (1 μ m) were stained with toluidine blue for orientation. Thin sections were then cut on an ultramicrotome, stained with uranyl acetate/lead citrate, and examined in an electron microscope. Numerous fields were studied to assess cell membranes and to look for various cytoplasmic constituents including lysosomes and Birbeck granules.

Results

Five out of the original 19 cases in this study met the criteria for HS. Of the excluded cases, nine could be reclassified, after an extensive immunohistochemical work-up, as anaplastic large cell lymphoma (three cases), diffuse large B-cell lymphoma (three cases), interdigitating dendritic cell sarcoma/tumor (two cases) and myeloid sarcoma (one case). The

remaining two tumors could not be categorized, demonstrating no specific immunophenotype, in addition to lacking CD45 immunoreactivity. As mentioned previously, further studies were not possible for three/19 specimens due to unavailable tissue blocks.

Clinical Findings

Available clinical data including clinical presentation, tumor size, stage, disease course, and clinical outcome are summarized in Table 2. There were three males and two females (*M:F* = 3:2) with ages ranging from 20 to 73 years (mean, 57.8 years). One case presented in the inguinal lymph node and four cases presented in extranodal sites including the colon (two cases), testis (one case), and palate (one case). Three patients were found to have stage IV disease on further work-up defined by involvement of multiple extralymphatic organs (Case 2), bone marrow (Case 3), or liver (Case 4). The colon was

involved in each of these cases resulting in abdominal pain as one of the presenting complaints. Bone marrow tissue was not available for histologic review. By report, none of the patients manifested a leukemic component, including those with high-stage disease.

Follow-up information (Table 2) was obtained in all cases and ranged from 2 months to 17 years. Although the number of patients in this study was small, the stage of disease appeared to correlate with the clinical course. The average length of survival among the three patients with known stage IV disease was 7 months. In contrast, Patient 1, who presented with localized extranodal disease involving the palate (stage I_E), remained disease free following his initial treatment and died of unrelated causes, 17 years after his initial diagnosis.

Tumor size also appeared to be an important predictor of clinical behavior. Each case that had poor survival, had a presenting tumor that was ≥ 3.5 cm in greatest dimension (range 3.5–9.5 cm). The median survival in these four cases was 6

Table 2 Clinical features

Case	Age/sex race	Presentation	Stage	Tumor (size)	Clinical course/treatment	Follow-up ^a
1	72M	<i>Ulcerating palatal nodule</i>	I _E	Palate lesion (2 cm)	Surgery ^b	DWOD
	Unknown	Local disease No systemic symptoms Past history of follicular lymphoma				17 years
2	69M	<i>Abdominal pain and testicular mass</i>	IV	Metastasis to forehead (3 cm) ^c	Unresponsive to chemotherapy and radiation therapy Progressive disease	DOD
	Black	Large peripancreatic mass (> 5 cm) invading stomach, pancreas and bowel Visceral adenopathy Cutaneous metastases				5 months
3	20F	<i>Ulcerating colonic mass</i>	IV	Sigmoid colon mass (6 cm)	Resolution of adenopathy and marrow involvement after chemotherapy Relapsed 8 months after initial diagnosis Unresponsive to further chemotherapy	DOD
	Caucasian	Abdominal pain with bloody diarrhea 30 lbs weight loss Retroperitoneal adenopathy Bone marrow involvement				15 months
4	55M	<i>Colonic mass causing intussusception</i>	IV	Colonic mass (9.5 cm)	Surgery (palliative)	DOD
	Caucasian	Abdominal adenopathy Hepatosplenomegaly			Rapid, progressive clinical course	2 months
5	73F	Cellulitis and associated adenopathy of inguinal area	Unk ^b	Inguinal lymph node (3.5 cm)	Surgery ^b	DOD
	Caucasian					3 months

^aDWOD = died without disease; DOD = died of disease.

^bFurther information not available.

^cPrimary abdominal mass seen radiographically but not biopsied.

months regardless of therapy. One of these patients (Case 3) achieved temporary remission following three cycles of chemotherapy (CHOP). Within 5 months, however, the patient relapsed with aggressive disease, which was unresponsive to further treatment. Aside from disease stage and tumor size, there were no readily identifiable clinical features that distinguished patients with localized disease and good prognosis, and those with extensive disease and aggressive clinical course.

Histologic Findings

The cases showed similar histologic features (see Figures 1–4). The extranodal cases showed infiltrative margins with focal destruction of the surrounding normal tissues. The lymph node specimen similarly showed near complete replacement of the node with focal extracapsular extension. All of the tumors demonstrated a diffuse growth pattern composed of large polygonal cells. Although the

cells were predominantly arranged in discohesive sheets, focal spindling was noted in three cases and was prominent in one (Case 4, see Figure 4). The neoplastic cells were large, with moderate to abundant eosinophilic cytoplasm, round to irregular nuclei with vesicular chromatin, and one to several distinct nucleoli. In addition, Cases 2 and 4 contained occasional cells with prominent nuclear grooves. Cellular atypia was mild to moderate with two cases showing pleomorphic and occasional anaplastic cells (Cases 3 and 4). Hemophagocytosis was identified within the tumor cells of three cases, however, this feature was only conspicuous in one (Case 1). Mitotic activity was highly variable throughout the five cases. Geographic necrosis was noted in the lymph node specimen (Case 5). Infiltrating neutrophils, lymphocytes, and occasional eosinophils were present and were focally abundant in the background in four of the cases.

Immunophenotypic Findings

The immunohistochemical features are listed in Table 3, and representative photomicrographs of Case 2 are depicted in Figures 2c–h. As one of the inclusion criteria, all specimens showed immunoreactivity for CD45, although expression was diffusely weak in two cases. Some variability in expression of histiocytic markers was noted; however, CD163 and CD68 consistently showed intense immunoreactivity. Lysozyme was weakly reactive in two cases, and MAC387 expression was somewhat inconsistent (2/5 positive, 1/5 focally positive, 2/5 negative). Scattered cells showed reactivity for factor XIIIa in two of the tumors. Whether these cells were a subset of the malignant population or were merely part of the non-neoplastic background was uncertain. S-100 was focally positive in four cases with one of these specimens showing more extensive areas of reactivity. All cases were negative for HMB-45, tyrosinase, and Melan A. Ki-67 immunoreactivity was variable, staining 1–15% of malignant nuclei. All remaining B- and T-cell, epithelial, melanocytic, and accessory/dendritic cell markers were uniformly negative.

Molecular Genetic Findings

Results of the IgH, TCR β , and TCR γ gene rearrangement studies are summarized in Table 4. Monoclonal IgH bands were identified in two cases and both IgH and TCR γ gene rearrangements were seen in one case. In all cases in which monoclonal bands were detected, B or T lymphocytes comprised <5%, and in most cases <1%, of the tissue cellularity. A small focus of residual lymph node was seen in Case 5. DNA was not amplifiable in Case 2, most likely due to low numbers of lymphocytes.

Figure 1 Case 1: histiocytic sarcoma of the palate with typical morphology. (a) Discohesive sheets of large, polygonal cells invading bone ($\times 100$); (b) Prominent hemophagocytosis within neoplastic cells (arrows) ($\times 400$).

Figure 3 Case 3: sigmoid colon tumor with nuclear pleomorphism, mitotic activity and occasional foci of hemophagocytosis (arrows) ($\times 100$).

Figure 4 Case 4: histiocytic sarcoma of the colon with area of prominent spindling ($\times 100$).

Electron Microscopic Findings

Ultrastructural analysis was carried out on three specimens (Cases 1, 3, and 5). The results in these cases were nonspecific and included scattered lysosomes within the cytoplasm, consistent with histiocytic origin. No Birbeck granules or desmosomes were observed. Occasional cytoplasmic processes were identified in Case 5 (inguinal lymph node); however, well-formed cellular junctions were not identified.

Table 3 Immunohistochemical results

Antibody	Case 1	Case 2	Case 3	Case 4	Case 5
CD45RB	wk+	+	+	wk+	+
CD3	—	—	—	—	—
CD5	—	—	—	—	—
CD20	—	—	—	—	—
CD79a	—	—	—	—	—
PAX5	—	—	—	—	—
CD163	+	+	+	+	+
CD68	+	+	+	+	+
Lysozyme	wk+	+	+	+	wk+
MAC387	+	+	—	—	foc+
Factor XIIIa	—	—	—	foc+	foc+
Fascin	+	+	+	+	+
S-100	foc+	—	foc+	foc+	+
HMB	—	—	—	—	—
Tyrosinase	—	—	—	—	—
Melan A	—	—	—	—	—
CD21	—	—	—	—	—
CD35	—	—	—	—	—
CD1a	—	—	—	—	—
CD30	—	—	—	—	—
CD34	—	—	—	—	—
MPO	—	wk+	—	wk+	—
CD56	—	—	—	—	—
Cytokeratin	—	—	—	—	—
EMA	—	—	—	—	—
LMP-1	—	—	—	—	—
Ki-67	5%	5%	15%	var 0–10%	15%

Immunoreactivity grading: + = strong, diffuse; wk+ = weak, diffuse; foc = focal; var = variable; — = negative.

Discussion

HSs are a problematic and controversial group of rare hematopoietic neoplasms. Clinically, these tumors appear to show some distinctive features including frequent extranodal presentation, poor prognosis^{19,20,33,41,50,51} and, as suggested by a recent study,⁵² in addition to the current one, a possible relationship between tumor size and poor prognosis. The evolution of the diagnosis of HS, as it is currently defined, is the product of continually improving diagnostic tools and more stringent diagnostic criteria. Despite its rarity, however, a growing number of reports have credibly documented the occurrence of these histiocytic tumors in nodal^{30,31,41} and a variety of extranodal sites including the gastrointestinal tract,^{35,37,39,41,42} skin,^{9,33,36,38,41} bone marrow,³⁴ spleen,³² and central nervous system.^{40,43,44}

In this study, we describe five neoplasms with the histopathologic features of true histiocytic origin. The diagnosis was based on morphology supported by an extensive immunophenotypic analysis that verified the expression in at least two histiocytic

Figure 2 Case 2: metastatic soft tissue mass of the forehead. (a) Histiocytic sarcoma diffusely infiltrating skeletal muscle ($\times 100$); (b) Many neoplastic cells with convoluted nuclear contours and occasional grooves, and virtual absence of a background of inflammatory cells ($\times 400$); (c) Representative immunohistochemical stains demonstrate reactivity for CD45, (d) CD163 (cytoplasmic and membranous, finely granular), (e) CD68, (f) Lysozyme (cytoplasmic, granular), and (g) MAC387; (h) No immunoreactivity for S-100 was identified ($\times 400$).

Table 4 Gene rearrangement studies

Case	Ig heavy chain	T-cell receptor β	T-cell receptor γ	Background lymphocytes	
				B-cells (CD20) (%)	T-cells (CD3) (%)
1	M	P	M	≤ 1	< 5
2	Not Amp	Not Amp	Not Amp	≤ 1	≤ 1
3	M	P	P	< 1	< 10
4	Not Amp	P	P	≤ 1	< 1
5	M	P	P	< 5	15

M = monoclonal band; P = polyclonal; Not Amp = not amplifiable.

antigens and excluded other large cell malignancies in the differential diagnosis. Although light microscopy alone is unreliable in establishing the diagnosis, the cases in the current study and review of literature describe similar histologic features consisting of discohesive large cells with pleomorphic nuclei and abundant cytoplasm.^{2,13,17,20,28,41,50–53} Malignant cytologic features with evidence of tissue invasion or destruction were constant findings in this study and are necessarily present in order to distinguish from benign histiocytic proliferations.^{18–20} Hemophagocytosis, a feature previously felt to be important in the diagnosis, may be present but is nonspecific^{29,54,55} and frequently is not prominent in bona fide cases.^{1,17,19,20,41,51} Likewise, this feature was inconspicuous in all but one of our cases.

The importance of immunophenotypic studies in the diagnosis of histiocytic neoplasms has been emphasized.^{13,17,51} However, establishing histiocytic lineage by special stains has historically been elusive. Markers once thought to be specific for histiocytic differentiation, such as alpha-1-antitrypsin, alpha-1-antichymotrypsin, lysozyme, and KP1, have been shown to have low specificity and may be positive in a wide variety of neoplasms, including a significant percentage of non-Hodgkin lymphomas.^{6,7,20,22–24,56,57} Moreover, CD68 (KP1) and alpha-1-antitrypsin immunoreactivity have been reported in melanoma with similar frequency to that of traditional melanoma markers (ie S-100, HMB-45), although the staining is usually not as intense.^{57,58} Monoclonal antibodies, such as PG-M1,⁵⁹ and more recently CD163,^{60–62} have demonstrated more restricted reactivity. In particular, CD163 has recently been studied in a variety of benign and malignant tissues and shows almost exclusive expression in cells of monocyte/macrophage lineage.^{26,63} A single study of cultured uveal melanomas showed expression of both CD68 and CD163 in the cultured cells although the tissue from the uveal melanoma was not immunoreactive with CD163.⁶⁴ In concordance with these studies, strong cytoplasmic immunoreactivity for CD163 was present in all of the cases in the current study, attesting to their histiocytic differentiation. This antigen has been identified as a hemoglobin scavenger receptor involved in the removal of free hemoglobin from the plasma as well

as the uptake of iron in tissue macrophages.^{25,62,65} In addition to this role in iron homeostasis, CD163 is also believed to have an anti-inflammatory function since it is rapidly upregulated in the presence of IL-10 and the glucocorticoid dexamethasone.^{25,61,62,65–67}

In addition to establishing histiocytic origin, immunohistochemistry plays an equally important and challenging exclusionary role in evaluating the differential diagnosis of histiocytic sarcoma. Our immunohistochemical analysis included an extensive panel of several epithelial, lymphoma, melanoma, and dendritic cell antibodies. Certain lymphoid markers, such as CD4 and CD22, were excluded since expression of these T- and B-cell markers may be expressed in some cells of myelomonocytic or histiocytic lineage.^{68–70} S-100, on the other hand, was a necessary component of the immunohistochemical analysis but may demonstrate variable results in histiocytic tumors. Immunoreactivity for this antibody in our study, therefore, required additional immunohistochemical stains to confidently exclude melanoma (ie melan A, HMB-45, tyrosinase), carcinoma (ie cytokeratin), and accessory/dendritic cell tumors (ie CD1a, CD21, CD35).^{2,51,71}

S-100 reactivity has been reported in benign and malignant histiocytic proliferations,^{19,41,43,51} although usually the degree of reactivity, as in the present study, is less than that seen in other accessory/dendritic cell tumors (ie Langerhans cell histiocytosis, interdigitating dendritic cell tumor).⁵¹ Immunohistochemistry and, if necessary, electron microscopy can usually delineate these lesions. However, antigen expression of some histiocytic and dendritic cell subsets appear similar using monoclonal antibodies, likely reflecting their close functional relationship.⁷² This phenomenon was observed in Case 5, which showed histologic, immunophenotypic, and ultrastructural characteristics of both HS and interdigitating dendritic cell sarcoma, though the preponderance of features favored the former. This specimen also showed the greatest degree of S-100 immunoreactivity as compared to the focal or absent staining noted in the other cases. As previously observed,^{37,41} this phenomenon may indicate that some histiocytic neoplasms show differentiation toward

antigen-presenting cells (ie interdigitating cells) or that these tumors may arise from a common cell of origin.

An interesting and novel finding in this study was the presence of focal factor XIIIa immunoreactivity. Although the identity of the positive cells as neoplastic or benign was difficult to determine, the expression of factor XIIIa in a subset of the histiocytes is not entirely unexpected as factor XIIIa expression has also been reported in Erdheim-Chester disease, a rare non-Langerhans' cell histiocytosis.⁷³ The significance of this finding and its possible role in the histogenesis of HSs is uncertain and requires investigation in a greater number of cases.

Since the mature tissue histiocyte is believed to be the benign counterpart of HS, CD45 (LCA) immunoreactivity has been recommended and was used in this study as a requirement to establish hematopoietic origin. It also allowed differentiation from morphologically similar soft-tissue neoplasms.²⁹ As not all histiocytes necessarily stain with CD45, however, the relevance of this criterion has been debated.⁷⁴ While CD45 immunoreactivity may not be an absolute requirement with the aid of additional supportive studies such as electron microscopy,⁴² differentiation from malignant fibrous histiocytoma (undifferentiated sarcoma) may be extremely difficult. These tumors are believed to arise from undifferentiated mesenchymal cells rather than histiocytes, and typically show pleomorphic and storiform growth patterns.^{75,76} Enzymatic and immunophenotypic studies^{77,78} have shown that the neoplastic cells in these tumors are negative for histiocytic markers but may contain numerous reactive histiocytes. Despite these subtle morphologic and immunophenotypic differences, clinical history may be the only means of differentiating HS from a metastatic soft-tissue malignancy involving lymph node.¹⁹ Although clinical history was somewhat limited in this study, the case involving lymph node showed immunophenotypic and ultrastructural features most compatible with a primary histiocytic tumor.

Another unresolved topic regarding the diagnosis of HS is the necessity and utility of molecular gene rearrangement studies. The difficulties are twofold: (1) there are no universal molecular genetic markers for detection of clonal histiocytic proliferations, and (2) the interpretation of IgH and TCR clonality studies can be problematic. While one would expect the histiocytic proliferations to lack clonal IgH or TCR rearrangements, in fact, these have been commonly reported in HSs^{27,28} and were also seen in this study. Monoclonal gene rearrangements were detected in three of five cases in this study, the remaining cases being not amplifiable for IgH. This phenomenon may represent a relatively common feature of malignant histiocytes, either lineage promiscuity related to the primitive nature of these cells^{79,80} or dual genotype in which some histiocytes

may abnormally retain the ability to rearrange the TCR and/or IgH genes despite phenotypically developing along histiocytic lines.²⁸ The former explanation is supported by the observation that histiocytic neoplasms occasionally arise in association with mediastinal germ-cell tumors^{81,82} and that IgH rearrangements have been previously reported in myeloid sarcoma.^{83,84}

Alternatively, cross-contamination from positive cases or 'pseudomonoclonality' due to extremely low numbers of lymphocytes may contribute to false positive molecular testing results.^{85,86} Although lymphocytes were sparse in the specimens in the present study, duplicate PCR testing decreases the likelihood of this pitfall. Finally, the presence of small clonal-like lymphoid populations, perhaps due to an immunologic response to the tumor,⁸⁷ is another consideration that may result in clonal gene rearrangements studies.

The possibility of the gene rearrangement studies defining tumor lineage as well as clonality, was also considered. However, classifying the tumors in our study as poorly differentiated lymphomas would imply a complete loss of all B- and T-cell antigens tested in our immunohistochemical panel, in addition to aberrant and exclusive expression of multiple histiocyte markers to include CD163. Therefore, we favor these neoplasms being of histiocytic origin based on the overwhelming phenotypic data and believe the results of the gene rearrangement studies most likely reflect an inherent property of the malignant histiocytes. Further studies involving microdissection of individual tumor cells followed by PCR amplification and sequence analysis may provide a more definitive explanation of these results.

Although the number of cases in this study is small, several conclusions may be drawn, which contribute to and further refine the clinicopathologic features of HS. The presentation of these rare hematopoietic tumors is more commonly extranodal, particularly involving the gastrointestinal tract. The clinical course is frequently aggressive with an associated high mortality rate. Stage of disease and possibly tumor size appear to be prognostically significant. Overlapping histologic, immunophenotypic, and ultrastructural features between HS and the dendritic cell neoplasms suggest a close relationship in the histogenesis of these tumors. Gene rearrangement studies remain a controversial issue but are probably not necessary to establish the diagnosis. Results must be interpreted in light of the clinical, morphologic, and immunophenotypic features. Continued investigation is necessary to fully understand the nature of these genetic findings and to further elucidate the pathogenesis of these neoplasms. Finally, the morphologic and immunophenotypic features are relatively uniform, however, an extensive panel of immunohistochemical markers is necessary to adequately exclude more common, poorly differentiated malignancies. Until

the recent characterization of CD163, histiocytic markers have typically demonstrated poor specificity and have confused the diagnosis of histiocytic neoplasms. CD163 is a sensitive and apparently restricted marker, with a rare exception, for identifying lesions of histiocytic lineage and is, therefore, important in the diagnosis of HS.

Disclaimer

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