Indeterminate Cell Tumor
A Rare Dendritic Neoplasm

Sherif A. Rezk, MD, Dominic V. Spagnolo, MD,† Russell K. Brynes, MD,‡
and Lawrence M. Weiss, MD§*

Abstract: Indeterminate cell tumor (ICT) is a rare neoplastic dendritic cell disorder that has been poorly defined due to its rarity and poorly understood histogenesis and pathogenesis. It is characterized by a proliferation of dendritic cells, which mimic Langerhans cells immunophenotypically (positive for CD1a and S-100 protein), but lack Birbeck granules characteristic of Langerhans cells. The clinical, morphologic, immunophenotypic, and ultrastructural features of 5 ICT cases are reported in an attempt to further define ICT and to examine the postulated relationship between indeterminate cells and Langerhans cells. Four of 5 patients were females, and 4 of 5 were older than 68 years. Three of 5 patients had cutaneous lesions, whereas 2 presented with cervical lymph node involvement. Two patients had a possible association with lymphoma: first patient had a history of progressive follicular lymphoma that led to patient's demise and the second patient had unexplained systemic lymphadenopathy and died 1 week after the biopsy. All 5 ICT cases expressed CD1a and S-100 protein, but lacked Langerin expression and Birbeck granules ultrastructurally. Interestingly, a t(14;18) was detected by fluorescence in situ hybridization in the ICT cells of the patient with previous follicular lymphoma and a monoclonal κ light chain gene rearrangement was detected by polymerase chain reaction in the patient with systemic lymphadenopathy. In both cases, there was no morphologic or immunophenotypic evidence of a concurrent B-cell lymphoma. In conclusion, ICT is a rare neoplasm that can occur de novo or in association with a B-cell lymphoma, possibly as a result of B-cell dedifferentiation caused by relatively unknown mechanisms. Finally, Langerin immunostaining may be used as a surrogate marker for the ultrastructural demonstration of Birbeck granules, the absence of which represents a strong diagnostic criterion for ICT.

Key Words: histiocytes, indeterminate, dendritic, Langerhans, Birbeck granules, Langerin

From the *Department of Pathology and Laboratory Medicine, University of California, Irvine (UCI), Irvine; ‡Department of Pathology, University of Southern California (USC), Los Angeles; §Department of Pathology, City of Hope National Medical Center, Duarte, CA; and †Anatomical Pathology, PathWest Laboratory Medicine WA (QEIIMC), Western Australia.
Correspondence: Lawrence M. Weiss, MD, Department of Pathology, City of Hope National Medical Center, 1500 East Duarte Road, Duarte, CA 91010 (e-mail: lweiss@coh.org).

Histiocytic and dendritic cell neoplasms are a relatively rare heterogeneous group of disorders that are characterized by the proliferation of 2 groups of cells: monocytes/macrophages (antigen-processing cells) and dendritic cells (antigen-presenting cells). It is believed that both groups are derived from the same bone marrow precursor cell population. Under the influence of various cytokines, these progenitor cells differentiate to become either antigen-processing or antigen-presenting cells.[10] The antigen-processing pathway involves the differentiation of the colony-forming unit (CFU)-granulocytic/monocytic cells into CFU-monocytic/dendritic cells, and then into CFU-monocytic cells. Subsequently, these progenitor cells differentiate into immature and finally into mature monocytes (Fig. 1).[1,17] The monocytes circulate through the blood and ultimately reside in various organs as tissue histiocytes and are referred to by several different designations, depending on their location.[1,17] A few examples include littoral cells of the venous sinuses, Kupffer cells of the liver, microglial cells of the brain, and type A synovial cells lining joints. The antigen-presenting pathway involves the differentiation of the CFU-monocytic/dendritic cells into CFU-dendritic cells and finally into immature dendritic cells, referred to by many as indeterminate cells. These indeterminate cells are thought to further differentiate into Langerhans cells and interstitial dendritic cells (Fig. 1).[1]

Indeterminate cell tumor (ICT) is a very rare disease that was first described in the early 1980s,[35,36] but has been omitted from most classifications of histiocytic and dendritic cell disorders mainly due to its rarity and poorly defined origin and pathogenesis. It is characterized by the presence of dendritic cells that immunophenotypically resemble Langerhans cells but lack Birbeck granules. Morphologically, there are some overlapping features with Langerhans cell histiocytosis (LCH) and non-Langerhans cell histiocytic disorders. In contrast to LCH, ICT usually shows a dense dermal infiltrate of dendritic cells with admixed lymphocytes but lacks significant eosinophilic infiltration or epidermotropism. ICT usually occurs in adults with no age or sex predilection, and predominantly involves the skin.[18] Clinically, ICT can occur as a solitary lesion or, less commonly, as multifocal disseminated lesions.[8,15,19] The disseminated lesions include multiple, asymptomatic, cutaneous papules usually occurring on the trunk, face, neck, or extremities.[8] Systemic symptoms and extracutaneous manifestations such as involvement of lymph
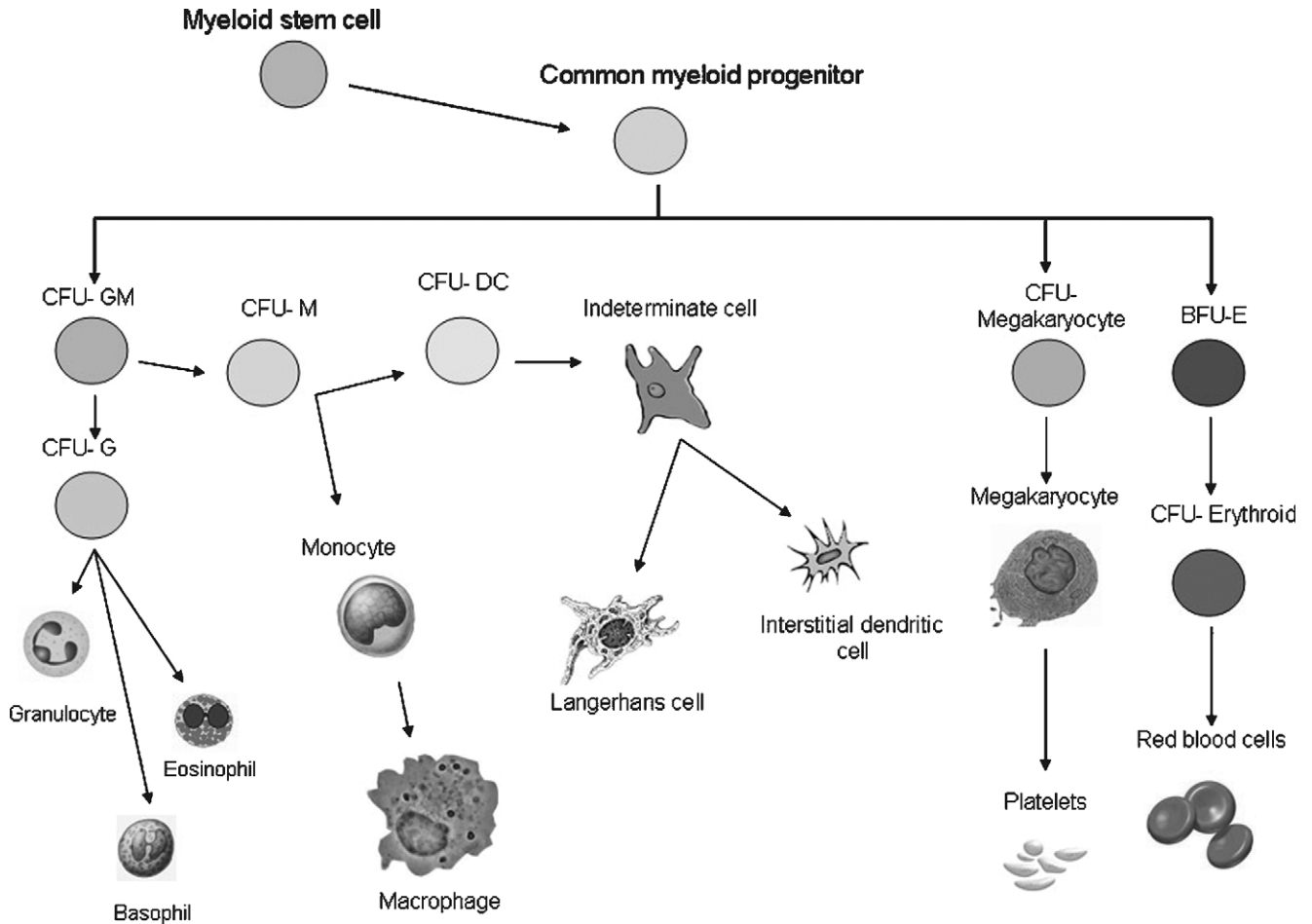
FIGURE 1. A projected scheme of histiocytic/dendritic cell maturation. BFU-E indicates burst-forming unit—erythroid; CFU-DC, colony-forming unit—dendritic cell; CFU-G, colony-forming unit—granulocyte; CFU-GM, colony-forming unit—granulocyte/ monocyte; CFU-M, colony-forming unit—monocyte.

nodes, bone, cornea, and genital areas have also been reported.[4,8,18,30] The clinical course of the disease is usually benign; however, the small number of ICT cases reported may not represent an accurate assessment of the clinical course of the disease. ICT has been rarely reported to be associated with other hematopoietic neoplasms including mast cell leukemia and acute myeloid leukemia, wherein evidence of common pathogenetic factors or actual disease transformation is lacking.[14,31]

A few cases of ICT have been reported in association with a prior or concurrent low-grade B-cell lymphoproliferative disorder.[30] Interestingly, recent studies performed on cases with simultaneous B-cell lymphoma and a dendritic/histiocytic neoplasm have reported the detection of identical heavy chain gene rearrangements or identical chromosomal aberrations demonstrated by fluorescence in situ hybridization (FISH) in both the neoplastic dendritic/histiocytic cells and lymphoma cells.[7,16] Another group has reported the detection of identical heavy chain gene rearrangements in both samples of a patient initially diagnosed with

precursor B-acute lymphoblastic leukemia with later development of a malignant histiocytic proliferation.[3] The constellation of these findings raises the possibility of a common progenitor for B lymphocytes and histiocytic/ dendritic cells.

Traditionally, histiocytic disorders have been divided into "X" (Langerhans) and "non-X" types. In the 2001 World Health Organization (WHO) Classification of Neoplasms of the Hematopoietic and Lymphoid tissues, histiocytic and dendritic cell neoplasms were classified according to their putative normal cellular counterparts.[12] ICT was not categorized as such in that classification due to its poorly understood nature and pathogenesis. Rather, it was classified under "dendritic cell sarcoma, not otherwise specified."[12] It was then included (as indeterminate cell histiocytosis) in the WHO Classification of Tumors of the Skin; however, it was described as a cutaneous neoplasm that is usually restricted to the skin.[5] In the forthcoming revision of the WHO Classification of Tumors of the Hematopoietic and Lymphoid Tissues (in press), ICT will be included in its own right. In the current paper, we report the

clinicopathologic features of 5 ICT cases in an attempt to better define this entity, and also examine the efficacy of Langerin immunostaining in the differential diagnosis.

MATERIALS AND METHODS

The cases included in this study were obtained from the pathology files of the Department of Pathology at City of Hope National Medical Center, Duarte, CA, and from the Department of Anatomical Pathology, Path-West Laboratory Medicine (Queen Elizabeth II Medical Center), Western Australia, Australia. Three of the cases were received as consult cases to City of Hope from outside hospitals. The ICT cases selected were obtained between the years 1994 and 2007. Two of the cases had 2 specimens derived from 2 different biopsies performed within the same year. Electron microscopy (EM) was performed on all 5 cases. A panel of immunohisto-chemical stains was performed, including antibodies to CD1a, S-100 protein, CD68, CD123, CD163, CD43, CD4, CD56, CD45, CD3, CD20, CD79a, PAX5, OCT-2, BOB-1, CD30, and Langerin. The specifications and titers of all the antibodies used are given in Table 1. A minority of the stains were performed in other laboratories. Routinely processed formalin-fixed paraffin-embedded tissue sections were used for immunohistochemical studies. Sections were mounted onto ChemMate capillary gap slides (Ventana Medical Systems Inc, Tucson, AZ), baked at 56°C for 60 minutes, deparaffinized with xylene, and rehydrated with ethanol to distilled water. Sections were deparaffinized in xylene and rehydrated in a graded ethanol series. Staining was performed using an auto-mated immunostainer (DAKO, Carpinteria, CA) followed by antibody detection using the DAKO EnVision + System and 3, 3-diaminobenzidine as a chromogen. The slides were counterstained with hematoxylin and cover slipped. Appropriate positive and negative tissue control samples were used throughout.

FISH analysis was performed on case 3 using a dual-fusion IGH/BCL2 probe (Vysis, Downer Grove, IL) on paraffin sections of the tumor mounted on glass slides. The slides were soaked in xylene to remove paraffin followed by 3 washes in 100% ethanol and 2 washes in distilled water. They were preheated in a 0.01 M trisodium citrate solution (pH 6) then heated again for 2 minutes in the microwave oven. The slides were washed in distilled water then incubated in a 2.5 mg/mL solution of pepsin in 0.01 N HCl at 37°C for 15 minutes, followed by a wash in phosphate-buffered saline. The nuclei were fixed by incubating the slides in a 37% formaldehyde solution for 5 minutes followed by a 5-minute wash in phosphate-buffered saline. A sequential dehydration in 70%, 90%, and 100% ethanol was then performed. The probes were applied and the slides incubated at 80°C for 10 minutes then hybridized at 37°C overnight. The slides were washed in 0.4 × standard sodium citrate/0.3%NP40 at 74°C for 2 minutes then in 2 × standard sodium citrate/0.1%NP40 at room temperature for 1 minute. The nuclei were stained with 4,6-diamidino-2-phenylindole and antifade was applied.

Polymerase chain reaction (PCR) studies were performed on 3 cases. Total cellular DNA was extracted from paraffin wax-embedded tissue. For immunoglobulin heavy chain gene rearrangement, 3 sets of primers were used as forward primers, as follows: framework 3a primer: 5′ TET-GAG GAC ACG GCT GTA TATTAC TGT 3′; framework 2a primer: 5′ HEX-TGG (A/G) TCCG(C/A) CAG (G/C)C(T/C) (T/C) CN GGG 3′; and framework 2b primer: 5′ FAM-GTC CTG CAG GC(C/T)C/T)CCGG(A/G) AA(A/G) (A/G)GT CTG GAG TGG 3′; and VLJHa as the reverse primer: 5′ CAC CTG AGG AGA CGG TGA CC3′. The amplified PCR products were visualized by capillary electrophoresis on an ABI310 genetic analyzer (PE Applied Biosystems, Foster City, CA). The amplified PCR products using identical, nonfluorescence primers subsequently were purified and subjected to direct DNA sequencing. For

TABLE 1. List of Antibodies

Name	Type	Clone No.	Titer	Manufacturer
Langerin/CD207	Monoclonal	12D6	1:100	Novocastra Laboratories Ltd, Newcastle, UK
CD1a	Monoclonal	O10	1:2	Beckman Coulter Inc, Fullerton, CA
CD68	Monoclonal	PG-M1	1:200	Dako Corporation, Carpinteria, CA
S100	Polyclonal	—	1:3000	Dako Corporation, Carpinteria, CA
CD43	Monoclonal	L60	1:10	Ventana Medical Systems Inc, Tucson, AZ
CD45	Monoclonal	2B11 + PD7/26	1:300	Dako Corporation, Carpinteria, CA
CD4	Monoclonal	1F6	1:15	Novocastra Laboratories Ltd, Newcastle, UK
CD123	Monoclonal	6H6	1:40	eBiosciences, San Diego, CA
CD163	Monoclonal	10D6	1:100	Novocastra Laboratories Ltd, Newcastle, UK
CD56	Monoclonal	123C3	1:100	Zymed Laboratories, S. San Francisco, CA
CD20	Monoclonal	L26	1:400	Dako Corporation, Carpinteria, CA
CD3	Polyclonal	—	1:200	Dako Corporation, Carpinteria, CA
CD79a	Monoclonal	JCB117	1:200	Dako Corporation, Carpinteria, CA
PAX5	Monoclonal	24	Neat	Cellmarque, Rocklin, CA
BOB-1	Polyclonal	—	1:800	SantaCruz Biotechnology
OCT-2	Monoclonal	OCT-207	1:50	Novocastra Laboratories Ltd, Newcastle, UK

immunoglobulin κ light chain gene rearrangement, primers for Fr3k and immunoglobulin κ light chain joint region 3 (CDR3) were used as follows: Fr3k 5′ TTC AG(C/T) GGCAGC GG(A/G) TCT GGG 3′ and JK 5′ CA(G/C) CTT(G/T)GTCCC(C/T)TG GCC GAA 3′. The amplified PCR products were electrophoresed in an 8% acrylamide gel and visualized with ethidium bromide staining. PCR on case 3 was performed in an outside laboratory where cellular DNA was extracted from paraffin wax embedded tissue using the QIAmp DNA MiniKit (Qiagen, Hilden, Germany). Consensus oligonucleotide primers for the V and J regions of the IgH gene were used for the detection of IgH gene rearrangements. PCR analysis of the t(14;18) translocation was carried out using primers annealing to IgH (JH) and BCL2 genes (MBR and mcr).

RESULTS

The clinical features of the 5 ICT cases are summarized in Table 2. Four of the 5 patients in our series were females. The mean age was 70 years (4 of 5 were older than 68 y). There was no known antecedent or subsequent associated malignancy in 3 patients. One of the remaining 2 patients (case 3) had a history of preceding stage IV follicular lymphoma with marginal zone differentiation. She eventually died of progressive and widespread lymphoma (postmortem examination was not performed). The other patient (case 4) presented with systemic lymphadenopathy and splenomegaly but unfortunately, she died 1 week after the biopsy was obtained. No autopsy was performed leaving us with no clear explanation for the lymphadenopathy and splenomegaly. Three patients had cutaneous ICT, whereas in 2, the ICT occurred intranodally in the absence of cutaneous involvement. All lesions were solitary.

Histologically, the 2 nodal ICT cases showed near-total effacement of the nodal architecture by a neoplastic infiltrate composed predominantly of large cells having copious, pale eosinophilic cytoplasm (Fig. 2). Nuclei were mostly oval with variably irregular contours, prominent eosinophilic nucleoli, and finely granular chromatin (Fig. 3). A few cells had elongated nuclei with occasional grooves running the length of the nucleus. Rare cells
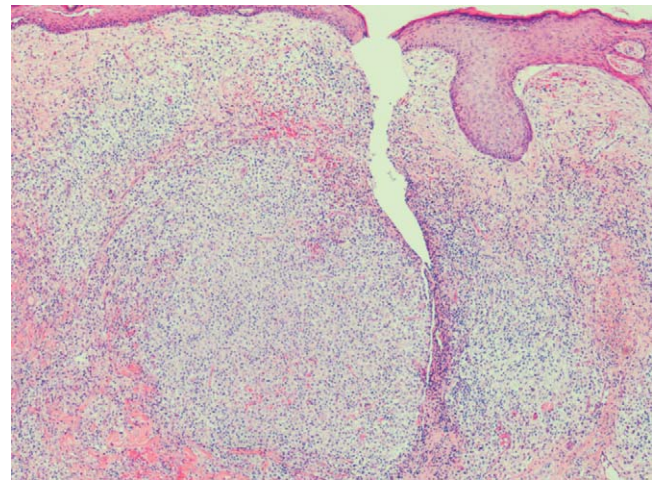

FIGURE 2. Low magnification image from case 5 shows a dense dermal infiltrate that has a nodular and diffuse pattern of involvement. The infiltrating cells appear to have copious amounts of cytoplasm. Scattered multinucleated cells are identified. No epidermal involvement is seen.

resembled Reed-Sternberg cells. The neoplastic cells infiltrated the nodes diffusely although occasional foci exhibited vague nodularity. The 3 cutaneous cases showed dense dermal infiltration. Occasional extension into the subcutaneous fat and minimal epidermotropism was identified in only 2 cases. Although the cytologic features were similar to those seen in the nodal cases, the cutaneous infiltrates exhibited greater variability in cell size and more pronounced nuclear irregularities including more frequent nuclear grooves. Eosinophils were sparse in all biopsy specimens. Two of the skin cases had 2 specimens derived from 2 different biopsies performed within the same year and both showed a similar morphologic and immunophenotypic picture.

The immunohistochemical results are summarized in Table 3. CD1a and S-100 stains were positive in all cases, although staining intensity and the number of positive cells varied between cases (Figs. 4, 5). Langerin staining performed on 4 specimens was negative. Two specimens contained scattered positive cells, probably

TABLE 2. Clinical Features of Indeterminate Cell Tumor Patients

| Case	Sex	Age (y)	Site of Involvement	Associated Clinical Findings and History
1	Female	68	Lymph node, left cervical	Chronic left submaxillary sialadenitis, no previous history of malignancy
2	Female	45	Skin, right upper arm	No known previous history of malignancy
3	Female	74	Skin, abdominal wall	Lesion at the end of a scar (site of previous lymph node biopsy), left groin follicular lymphoma, squamous and basal cell carcinomas at sun-exposed areas, splenomegaly, died of progressive lymphoma
4	Female	79	Lymph node, right cervical	Systemic lymphadenopathy and splenomegaly, no previous history of malignancy, patient died 1 wk after the biopsy with no autopsy performed
5	Male	87	Skin, left cheek	Previous left postauricular skin excision diagnosed as atypical Langerhans cell histiocytosis
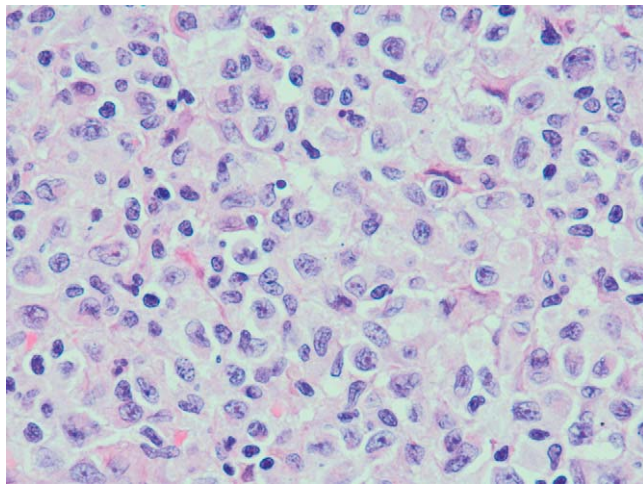
FIGURE 3. High magnification image from case 5 shows that the neoplastic cells have irregular nuclear contours, open chromatin, and copious amounts of eosinophilic cytoplasm. Rare cells exhibit longitudinal grooves. Scattered small lymphocytes are admixed within the infiltrate.

attributable to the staining of reactive Langerhans cells (Fig. 6). CD68 had a variable expression pattern in 3 of the 5 cases. The intensity and uniformity of expression was variable but all cases showed a positivity that was mostly restricted to the paranuclear area along with scattered admixed histiocytes that showed diffuse cytoplasmic staining. Other dendritic and histiocytic stains such as CD43, CD163, CD123, and CD4 showed variable and inconsistent results among the ICT cases. Despite the fact that CD163 is normally expressed by functionally mature histiocytes, the lesional cells in 3 of 5 cases showed focal expression of CD163 along with scattered admixed positive histiocytes. The only stain other than CD1a and S-100 protein that was consistently positive in all ICT cases was focal or diffuse CD45 expression. All cases contained scattered positively stained small lymphocytes.

All 5 ICT cases were evaluated by EM (Table 3). The neoplastic cells exhibited dendritic morphology. After intensive scrutiny, none revealed Birbeck granules (Fig. 7). The cytoplasm was generally abundant and organelle-rich but lacked specific features. In the Golgi region of some cells, small lysosomal granules could occasionally be found but there was no phagocytosis or

TABLE 3. Results of Electron Microscopy, Molecular Studies, and Immunohistochemistry

	ICT Case 1	ICT Case 2	ICT Case 3	ICT Case 4	ICT Case 5
Electron microscopy for Birbeck granules	Negative	Negative	Negative	Negative	Negative
Fluorescence in situ hybridization for t(14;18)	N/A	N/A	Positive in 78% of lesional cells	N/A	N/A
Polymerase chain reaction for B-cell gene rearrangement	N/A	N/A	Insufficient DNA for meaningful assessment	κ monoclonal light chain present with no heavy chain detected	No monoclonality detected
Langerin	N/A	Negative (rare cells positive)	Negative (rare cells positive)	Negative	Negative
CD1a	Positive	Positive	Positive (focal)	Positive	Positive
S-100	Positive	Positive (subset)	Positive	Positive (small subset)	Positive
CD45	Weakly positive	Positive	Positive	Positive	Positive (focal)
CD123	N/A	Negative	Negative	Positive	Negative
CD163	N/A	Positive (focal)	Negative	Positive (focal)	Positive (focal)
CD68	Negative	Focally positive (punctate and restricted to the Golgi zone)	Negative	Positive (punctate)	Positive (punctate and restricted to the Golgi zone)
CD56	N/A	Positive	Negative	Positive	Positive
CD43	Negative	Positive	Negative	Positive	Weakly positive
CD4	N/A	Negative	Positive	Positive	Negative
CD30	Negative	Negative	Negative	Negative	Negative
CD3	Negative	Negative	Negative	Negative	Negative
CD20	Negative	Negative	Negative	Negative	Negative
CD79a			Negative	Rare small B cells positive, lesional cells negative	
PAX5			Negative	Scattered small B cells positive	
OCT-2			Weakly positive in < 10% of the cells		
BOB-1			Negative		

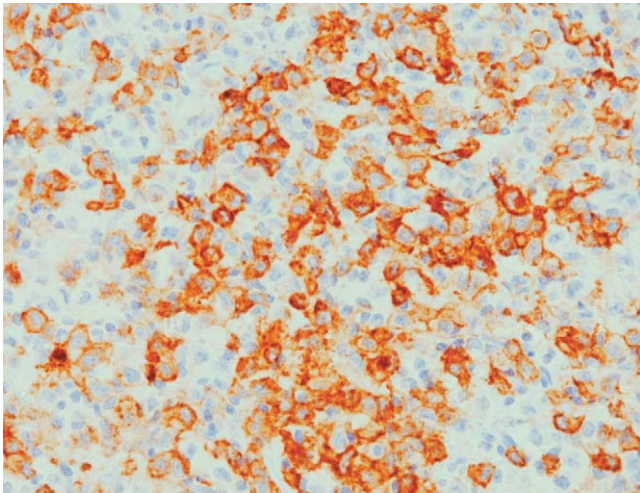ICT includes indeterminate cell tumor; N/A, not available.

FIGURE 4. The image from case 5 shows positive staining of the neoplastic cells to CD1a stain. The background shows admixed small to medium-sized lymphocytes that are negative.
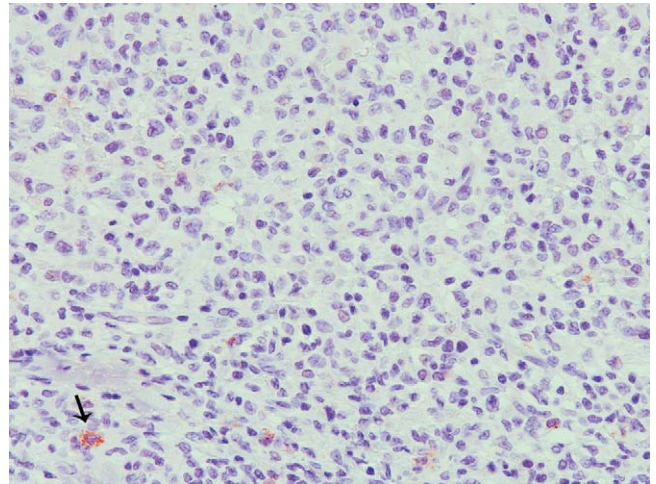

FIGURE 6. The neoplastic cells in case 5 are clearly negative for Langerin. The arrow points to a rare positive cell, which probably represents a bystander Langerhans cell.

endocytosis. Nuclei were sometimes indented or convoluted. Cell surface morphology varied and included cells demonstrating parallel arrays of flattened lamellipodia between cells, to others that formed broader, interleaving processes. There were no defined intercellular junctions. Rarely, foci of apposing subplasmalemmal linear densities were present between cell processes.

B-cell gene rearrangement studies using PCR were attempted in 3 cases (cases 3, 4, and 5). The DNA was too degraded for meaningful assessment in case 3, whereas the attempts on the other 2 cases were successful. In case 4 (previous history of unexplained systemic adenopathy), a monoclonal κ gene rearrangement was detected without monoclonal Ig heavy chain gene rearrangement. In case 5

(no previous history of lymphoma), only polyclonal Ig gene rearrangements were obtained. FISH studies using a dual-fusion IGH/BCL2 probe were also performed on case 3 (skin lesion in a patient with history of progressive follicular lymphoma). The IGH-BCL2 fusion associated
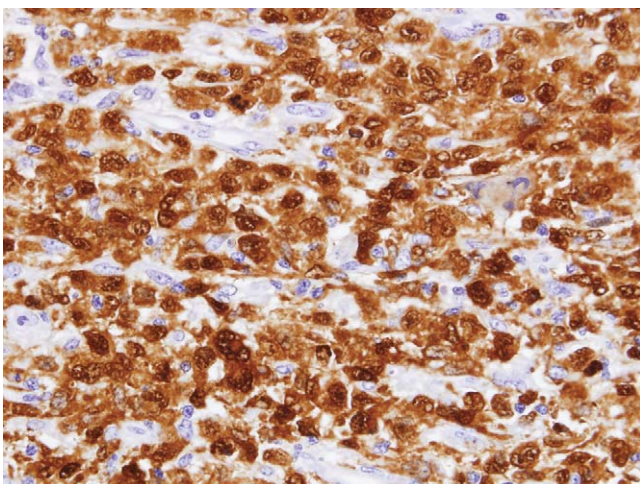

FIGURE 5. The image from case 3 shows positive staining of the neoplastic cells to S-100 stain. The background shows scattered small lymphocytes along with few endothelial cells that are negative.
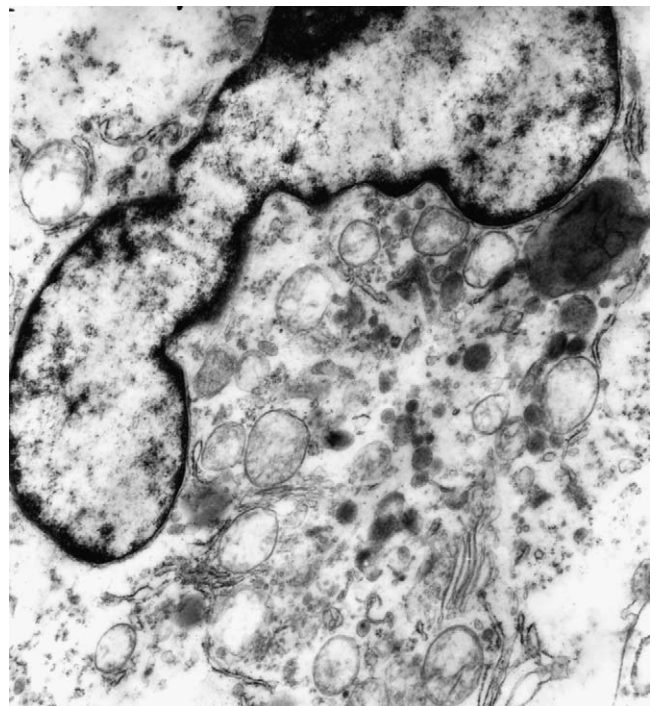

FIGURE 7. The electron micrograph from case 3 shows a representative indeterminate cell tumor with an eccentric, indented nucleus, a well-developed paranuclear Golgi, and several Golgi-associated vesicles and small, dense lysosomal granules. There are no Birbeck granules.
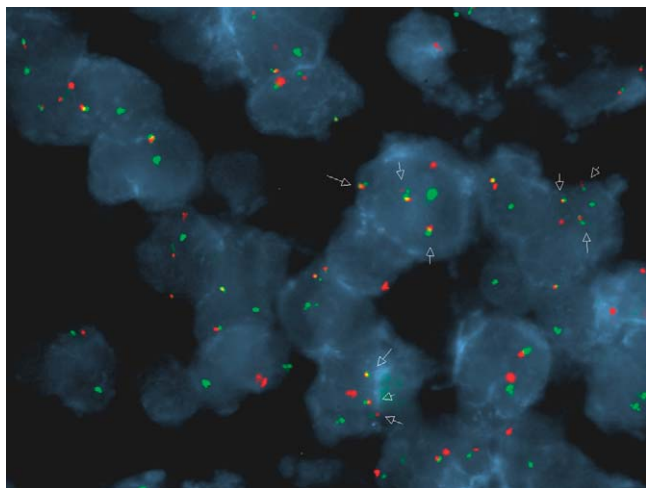
FIGURE 8. The fluorescence in situ hybridization picture from case 3 shows lesional indeterminate cell tumor in interphase. The IGH sequences on 14q32 (green) are juxtaposed to the bcl-2 sequences on 18q21 (red) to yield an orange/yellow fusion signals (arrowed), which indicates the presence of t(14;18). Extra fusion products are also identified.

with t(14;18) was detected in 78% of the cells (Fig. 8). Extra fusion products were also detected in some of the cells, which could possibly represent extra copies of der14 or der18. The molecular results are summarized in Table 3.

DISCUSSION

ICT is a rare neoplastic disorder that has been historically characterized by a similar immunophenotype and, in some cases, a similar morphology to that of LCH. Ultrastructurally, ICT cells are rich in cytoplasmic organelles and show numerous dendritic processes that interdigitate with those of adjacent cells; however, they lack Birbeck granules, which is the main distinguishing feature between ICT and LCH.[33] Langerhans cells are located predominantly in the epidermis and in the mucosal surfaces of various organs. They characteristically express CD1a and possess distinctive granules that were first described by Birbeck et al in 1961.[2] The function of Birbeck granules, which are considered to be a specific marker of Langerhans cells, remains unknown although some have suggested a potential role in antigen capture.[29] Birbeck granules are thought to originate from the plasmalemma by invaginations of endocytic structures, their formation mediated by Langerin/CD207, a type II transmembrane protein.[27,29] Langerin expression has been reported to be restricted to Langerhans cells using PCR analysis and several immunophenotypic techniques.[27,28] Indeterminate cells are considered by many to be pre-Langerhans cells, as they express CD1a and S-100 but lack Birbeck granules and theoretically should be negative for Langerin expression.

The origin and function of indeterminate cells have been the subject of numerous debates and speculations, which may have prevented the disorder from acquiring distinction as a specific entity. Rowden and colleagues[20] proposed in 1979 that indeterminate cells represent immature forms of Langerhans cells that complete their acquisition of Birbeck granules on migration to the epidermis. Others have suggested that they are immune accessory antigen-presenting cells that undergo dermal arrest during their physiologic trafficking from the skin to the paracortex of lymph nodes and thus give rise to ICT.[19,25] The possibility of these cells being accessory antigen-processing cells in immune-mediated inflammatory lesions such as contact dermatitis and scabies has also been hypothesized.[11,19,21] The immunophenotypic similarity between ICT and LCH may indicate that indeterminate cells are either a preceding or a succeeding event in the development of Langerhans cells. S-100 protein is a nonspecific marker of histiocytic neoplasms and has been linked to many entities, whether epithelial, melanocytic, neural, or hematopoietic.[9] On the other hand, CD1a has a more limited scope of expression but is not specific for LCH as it can be detected in precursor T-acute lymphoblastic leukemia and may be expressed in some cases of interdigitating dendritic cell tumor.[9] Given that ICT consistently expresses both antigens, one can reasonably conclude that there is a close relationship between ICT and LCH.

Despite the lack of definite literature support for sex or age predilection in ICT, 4 of our 5 patients were females, and most were elderly (mean age was 70 y, and 4 of 5 patients were older than 68 y). The reason for this difference is not clear, and does not warrant speculation because of the small number of cases studied. Three of our patients presented with solitary skin lesions, and in 2 of these, other solitary skin lesions subsequently developed. The remaining 2 patients presented with cervical lymph node involvement. In all 5 cases, the histologic appearances were relatively uniform, with minimal cytologic variation. EM performed on all cases failed to reveal Birbeck granules in any case. Langerin immunostaining was performed on 4 of the 5 cases and, as expected, no expression was noted except in a very small subset of cells that were interpreted as bystander Langerhans cells and not part of the neoplastic infiltrate, based on their morphology and distribution. These scattered Langerin-positive cells were noted in both the skin and nodal cases. The lack of Langerin staining in ICT cases correlates with the absence of Birbeck granules ultrastructurally. In contrast, LCH cases are strongly and diffusely positive for Langerin.[24,34]

The identification of scattered cells in all ICT cases that possess nuclear grooves reminiscent of Langerhans cells and the detection of scattered cells that express Langerin by immunohistochemistry may indicate the presence of bystander Langerhans cells within the neoplastic ICT infiltrate. These bystander cells may represent reactive cells in their normal location although one cannot discount the possibility that they are neoplastic indeterminate cells, which may have escaped the putative maturational arrest in ICT and have

proceeded to more differentiated Langerhans cells. The reported loss of Birbeck granules in tissue culture of some LCH cases due to a naturally occurring point mutation in the Langerin gene[13,26,32] may further indicate that indeterminate cells and Langerhans cells derive from a common precursor and represent different evolutionary/maturational stages. The presence of scattered Langerin-positive cells in 2 of 4 cases examined associated with the total absence of Birbeck granules ultrastructurally in all cases may simply indicate a sampling problem, which is inherent in ultrastructural examination. However, it would be interesting to ascertain immunoultrastructurally whether Langerin expression may indeed occur at the subcellular level in the absence of Birbeck granules.

Molecular tests in our study revealed the presence of the t(14;18) by FISH (case 3) and a monoclonal κ light chain gene rearrangement by PCR (case 4) in the 2 patients with a history of follicular lymphoma, and unexplained systemic lymphadenopathy, respectively. Morphologic and immunophenotypic review of both cases showed no evidence of lymphoma or increased number of B lymphocytes coexisting with the ICT, which probably indicates that the molecular abnormalities are present within lesional ICT cells. These results are in concordance with what others have reported in some cases of mature and immature B-cell neoplasms associated with simultaneous or later development of various histiocytic/dendritic cell tumors.[3,7,16,30] This may be suggestive of the presence of a common precursor for the B cells and the histiocytic/dendritic cells or the presence of a factor that causes loss of the B-cell identity and alters their normal pathway of differentiation and maturation. Developmental plasticity of B cells along with loss of the B-cell transcription factor PAX5 may be the culprit in the process of dedifferentiation (or transdifferentiation) of B cells. Under normal circumstances, mature B cells differentiate into either memory B cells or plasma cells after their passage through the germinal center.[22] The transcription of PAX5 is down-regulated in the event of terminal differentiation of B cells into plasma cells, which is believed to be initiated in response to antigen stimulation of the B-cell receptor (BCR).[6,23] On the basis of the fact that the loss of PAX5 in the context of BCR signaling results in forward differentiation of mature B cells into plasma cells, some investigators have demonstrated that deletion of PAX5 in the absence of BCR signaling leads to in vivo dedifferentiation of B cells into uncommitted hematopoietic progenitors in the bone marrow.[6] PAX5-deficient progenitors may differentiate into myeloid cells and T cells, but not into B cells.[6] This hypothesis is further supported by the reported in vitro transdifferentiation of mature B cells into macrophages induced by the inhibition of PAX5 and by the enforced expression of the transcription factors C/EBP α and β.[37]

In conclusion, despite the rarity of ICT and the lack of evidence solidifying the many hypotheses related to the origin and pathogenesis of the disease, ICT should be considered as a clincopathologic entity. It can occur de novo, in which case the disease is usually limited to the skin, or less commonly, lymph node, and the prognosis is good; or it may occur in the course of a previous or a concurrent B-cell lymphoma, possibly due to an underlying factor causing B-cell dedifferentiation, in which case the prognosis is due that of the lymphoma. Additional studies will be required to assess whether these latter cases are identical to the more isolated form of the neoplasm. While recognizing that very few cases have been thoroughly studied, the absence of Langerin expression, at the present time, should be a requirement for diagnosis and may serve as a surrogate marker for the absence of Birbeck granules ultrastructurally. Langerin immunostaining is a faster and aless expensive alternative than EM examination for both ICT and LCH cases.

REFERENCES
1. Arceci RJ. The histiocytoses: the fall of the Tower of Babel. Eur J Cancer. 1999;35:747–767; discussion 767–769. Review.
2. Birbeck MS, Breathnach AS, Everall JD. An electron microscope study of basal melanocytes and high-level clear cells (Langerhans cells) in vitiligo. J Invest Dermatol. 1961;37:51–63.
3. Bouabdallah R, Abéna P, Chetaille B, et al. True histiocytic lymphoma following B-acute lymphoblastic leukaemia: case report with evidence for a common clonal origin in both neoplasms. Br J Haematol. 2001;113:1047–1050.
4. Calatayud M, Güell JL, Gris O, et al. Ocular involvement in a case of systemic indeterminate cell histiocytosis: a case report. Cornea. 2001;20:769–771.
5. Caputo R, Berti E. Indeterminate cell histiocytosis. In: LeBoit PE, Burg G, Weedon D, et al, eds. World Health Organization Classification of Tumors. Pathology and Genetics of Skin Tumors. Lyon: IARC Press; 2006:220–221.
6. Cobaleda C, Jochum W, Busslinger M. Conversion of mature B cells into T cells by dedifferentiation to uncommitted progenitors. Nature. 2007;449:473–477.
7. Feldman AL, Arber DA, Pittaluga S, et al. Clonally related follicular lymphomas and histiocytic/dendritic cell sarcomas: evidence for transdifferentiation of the follicular lymphoma clone. Blood. 2008 [Epub ahead of print].
8. Ferran M, Toll A, Gilaberte M, et al. Acquired mucosal indeterminate cell histiocytoma. Pediatr Dermatol. 2007;24:253–256.
9. Frater JL, Kling CW, Obadiah JM, et al. Histiocytic sarcoma with secondary involvement of the skin and expression of CD1a: evidence of indeterminate cell differentiation? J Cutan Pathol. 2006;33:437–442.
10. Gonzalez CL, Jaffe ES. The histiocytoses: clinical presentation and differential diagnosis. Oncology (Williston Park). 1990;4:47–60; discussion 60, 62.
11. Hashimoto K, Fujiwara K, Punwaney J, et al. Post-scabetic nodules: a lymphohistiocytic reaction rich in indeterminate cells. J Dermatol. 2000;27:181–194.
12. Jaffe ES. Histiocytic and dendritic cell neoplasms: Introduction. In: Jaffe ES, Harris NL, Stein H, et al, eds. World Health Organization Classification of Tumors. Pathology and Genetics of Tumors of Hematopoietic and Lymphoid Tissues. Lyon: IARC Press; 2001.
13. Kissenpfennig A, Aït-Yahia S, Clair-Moninot V, et al. Disruption of the langerin/CD207 gene abolishes Birbeck granules without a marked loss of Langerhans cell function. Mol Cell Biol. 2005;25:88–99.

14. Kolde G, Bröcker EB. Multiple skin tumors of indeterminate cells in an adult. *J Am Acad Dermatol.* 1986;15:591–597.
15. Levisohn D, Seidel D, Phelps A, et al. Solitary congenital indeterminate cell histiocytoma. *Arch Dermatol.* 1993;129:81–85.
16. Magni M, Di Nicola M, Carlo-Stella C, et al. Identical rearrangement of immunoglobulin heavy chain gene in neoplastic Langerhans cells and B-lymphocytes: evidence for a common precursor. *Leuk Res.* 2002;26:1131–1133.
17. Onciu M. Histiocytic proliferations in childhood. *Am J Clin Pathol.* 2004;122(suppl):S128–S136. Review.
18. Ratzinger G, Burgdorf WH, Metze D, et al. Indeterminate cell histiocytosis: fact or fiction? *J Cutan Pathol.* 2005;32:552–560.
19. Rosenberg AS, Morgan MB. Cutaneous indeterminate cell histiocytosis: a new spindle cell variant resembling dendritic cell sarcoma. *J Cutan Pathol.* 2001;28:531–537. Review.
20. Rowden G, Phillips TM, Lewis MG. Ia antigens on indeterminate cells of the epidermis: immunoelectron microscopic studies of surface antigens. *Br J Dermatol.* 1979;100:531–542.
21. Saijo S, Hara M, Kuramoto Y, et al. Generalized eruptive histiocytoma: a report of a variant case showing the presence of dermal indeterminate cells. *J Cutan Pathol.* 1991;18:134–136.
22. Shaffer AL, Rosenwald A, Staudt LM. Lymphoid malignancies: the dark side of B-cell differentiation. *Nat Rev Immunol.* 2002;2:920–932. Review.
23. Shapiro-Shelef M, Calame K. Regulation of plasma-cell development. *Nat Rev Immunol.* 2005;5:230–242. Review.
24. Sholl LM, Hornick JL, Pinkus JL, et al. Immunohistochemical analysis of langerin in Langerhans cell histiocytosis and pulmonary inflammatory and infectious diseases. *Am J Surg Pathol.* 2007;31:947–952.
25. Sidoroff A, Zelger B, Steiner H, et al. Indeterminate cell histiocytosis: a clinicopathological entity with features of both X- and non-X histiocytosis. *Br J Dermatol.* 1996;134:525–532. Review.
26. Teunissen M, Wormmeester J, Krieg S, et al. Human epidermal Langerhans cells undergo profound morphologic and phenotypical changes during in vitro culture. *J Invest Dermatol.* 1990;94:166–173.
27. Valladeau J, Duvert-Frances V, Pin JJ, et al. The monoclonal antibody DCGM4 recognizes Langerin, a protein specific of Langerhans cells, and is rapidly internalized from the cell surface. *Eur J Immunol.* 1999;29:2695–2704.
28. Valladeau J, Ravel O, Dezutter-Dambuyant C, et al. Langerin, a novel C-type lectin specific to Langerhans cells, is an endocytic receptor that induces the formation of Birbeck granules. *Immunity.* 2000;12:71–81.
29. Valladeau J, Dezutter-Dambuyant C, Saeland S. Langerin/CD207 sheds light on formation of Birbeck granules and their possible function in Langerhans cells. *Immunol Res.* 2003;28:93–107. Review.
30. Vasef MA, Zaatari GS, Chan WC, et al. Dendritic cell tumors associated with low-grade B-cell malignancies. *Am J Clin Pathol.* 1995;104:696–701.
31. Vener C, Soligo D, Berti E, et al. Indeterminate cell histiocytosis in association with later occurrence of acute myeloblastic leukaemia. *Br J Dermatol.* 2007;156:1357–1361.
32. Verdijk P, Dijkman R, Plasmeijer EI, et al. A lack of Birbeck granules in Langerhans cells is associated with a naturally occurring point mutation in the human Langerin gene. *J Invest Dermatol.* 2005;124:714–717.
33. Weiss LM. Histiocytic and dendritic cell proliferations. In: Knowles DM, ed. *Neoplastic Hematopathology.* Vol 2. Philadelphia: Lippincott Williams & Wilkins; 2001:1837.
34. Weitzman S, Jaffe R. Uncommon histiocytic disorders: the non-Langerhans cell histiocytoses. *Pediatr Blood Cancer.* 2005;45:256–264. Review.
35. Winkelmann RK, Hu CH, Kossard S. Response of nodular non-X histiocytosis to vinblastine. *Arch Dermatol.* 1982;118:913–917.
36. Wood GS, Hu CH, Beckstead JH, et al. The indeterminate cell proliferative disorder: report of a case manifesting as an unusual cutaneous histiocytosis. *J Dermatol Surg Oncol.* 1985;11:1111–1119.
37. Xie H, Ye M, Feng R, et al. Stepwise reprogramming of B cells into macrophages. *Cell.* 2004;117:663–676.