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ORIGINAL RESEARCH

Cytologic and immunocytochemical characterization of feline progressive histiocytosis

Nazaré Pinto da Cunha[1], Gabriele Ghisleni[1], Fabia Scarampella[2], Fabrizio Fabbrini[3], Monica Sforna[4], Luisa Cornegliani[1], Mario Caniatti[1], Giancarlo Avallone[1], Peter Moore[5], Paola Roccabianca[1]

[1]Department of Veterinary Sciences and Public Health, University of Milan, Milan, Italy; [2]Studio Dermatologico Veterinario, Milan, Italy; [3]Clinica Veterinaria Papiniano, Milan, Italy; [4]Department of Veterinary Medical Sciences, University of Perugia, Perugia, Italy; and [5]Department of Pathology, Microbiology and Immunology, School of Veterinary Medicine, University of California at Davis, Davis, CA, USA

Key Words
Cats, CD1, histiocytic disorders, immunocytochemistry, skin

Correspondence
Nazaré Pinto da Cunha
CEDIVET, Rua de Antero de Quental, 991, 2°D, 4200-071 Porto, Portugal
E-mail: nesmaria@msn.com

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Background: Feline Progressive Histiocytosis (FPH) is a cutaneous dendritic cell neoplasm characterized by slow progression and spread to internal organs in the terminal stage. FPH is often misdiagnosed as an inflammatory reaction and has not been fully characterized from a cytologic diagnostic perspective.
Objectives: The purpose of the study was to characterize the cytologic and immunocytochemical aspects useful for FPH diagnosis.
Methods: Fine-needle aspiration cytologic samples of 5 cases of FPH confirmed by skin biopsy and necropsy were evaluated. Immunocytochemistry with antibodies recognizing CD1a, CD1c, CD3, CD11b, CD18, CD21, and MHCII was performed on air-dried, acetone-fixed smears. E-cadherin expression was assessed on paraffin-embedded skin biopsies. Transmission electron microscopy (TEM) was performed in one case.
Results: Main cytologic findings on variably cellular samples were characterized by single to cohesive large, round to polygonal cells with intermediate to low N/C ratio, abundant clear homogeneous cytoplasm, and round to oval nuclei with rare bi- to multinucleated atypical cells, associated with low numbers of small lymphocytes and/or neutrophils. Neoplastic cells expressed CD1a, CD1c, CD11b, CD18, and MHCII. Anti-CD3 antibodies identified reactive T cells admixed with the neoplastic cells. E-cadherin expression was observed in all but one case. TEM failed to identify Birbeck granules in one case.
Conclusions: FPH is a distinctive neoplastic lesion composed of nonphagocytizing histiocytes variably admixed with neutrophils and small mature lymphocytes. Immunocytochemical analysis with CD1 is mandatory to confirm a dendritic cell origin. Immunocytochemistry and cytomorphology allowed the specific and rapid diagnosis of FPH on cytologic samples.

Introduction

Histiocytic proliferative diseases embrace a range of diseases with different clinical presentation and behavior. These diseases derive from either macrophages or various subtypes of dendritic cells (DC)[1], which are not easily distinguishable in routine cytology and histopathology. Characterization of histiocytic disorders, other than by morphology, and clinical presentation and behavior, requires immunophenotypic analysis to adequately identify the specific condition.

In dogs, several histiocytic disorders with DC or macrophage origin have been described including inflammatory and reactive forms[2–4], as well as neoplastic conditions.[5–10] Feline histiocytic diseases of DC or macrophage origin have been rarely reported and include Feline Progressive Histiocytosis (FPH)[11], Feline Pulmonary Langerhans Cell Histiocytosis (FPLCH)[12], and Feline Histiocytic Sarcoma (FHS), either localized[13–17] or disseminated/systemic.[18–23]

FPLCH is characterized by progressive respiratory failure due to primary pulmonary infiltration by

histiocytes, and is invariably fatal.[12] In this condition, neoplastic histiocytes express CD18 and E-cadherin, while in transmission electron microscopy, Birbeck granules were identified in the cytoplasm, indicating a Langerhans cell (LC) origin.[12]

Disseminated FHS, also often termed malignant histiocytosis, is most frequently characterized by involvement of the spleen, liver, and lymph nodes.[19–23] Localized forms have been documented in the skin[15], spleen[13], and central nervous system.[14,16] As in dogs, FHS has an aggressive clinical course with poor prognosis.[7,8] Morphologic features resemble those of canine histiocytic sarcoma (HS), including the finding of neoplastic cells with marked cytologic atypia. The expression of CD1, CD18, and MHC II is consistent with a DC origin, but the lack of E-cadherin expression has generally been considered exclusive for an LC origin.[7,11]

FPH has been recently described as a primary nodular skin disease that evolves into a fatal condition, characterized by invasion of regional lymph nodes and internal organs by neoplastic dendritic cells. It is considered a rare disease with no breed or age predilection, with a slight predilection for females. The typical clinical presentation of FPH is the presence of nonpruritic, nonpainful, single to multiple nodules or papules, that increase in size, may coalesce to large plaques, and tend to ulcerate. Lesions develop more frequently on extremities and head, and remain limited to the skin for a variable period of time. Some cats develop lesions in lymph nodes and internal organs including the lungs, kidneys, spleen, and liver, culminating in spontaneous death or euthanasia in a time lapse varying from one month to 3 years (average 13.4 months).[11] Conclusive diagnostic confirmation is based on histopathology and immunohistochemistry.

In cutaneous nodular diseases, cytologic analysis is frequently the first and least invasive step in the diagnostic process. Characterization of the cytomorphologic and immunocytochemical features of FPH could allow a prompt diagnosis of the disease. Additionally, the most useful marker to demonstrate a DC origin, CD1, cannot be applied to formalin-fixed samples, requiring the use of frozen tissues, thus complicating biopsy specimen collection and handling. A more practical and fast alternative is immunophenotypic evaluation of adequately cellular cytologic samples.

The aim of this report was to describe cytologic features of FPH, and to evaluate the feasibility of characteristic immunophenotypic expression testing of this tumor in cytologic samples with the ultimate purpose of establishing a prompt and reliable diagnostic approach to FPH.

Materials and Methods

Sample collection

Multiple cytologic samples from 5 cats presenting with cutaneous nodules were submitted to the Department of Veterinary Pathology, Hygiene and Public Health of the University of Milan, Italy, for a second opinion and immunocytologic studies, with the request to confirm or exclude the diagnosis of FPH. Samples were collected from skin lesions by fine-needle aspiration and were air-dried. One smear from each cat was stained with May–Grünwald–Giemsa (Merck, Darmstadt, Germany). Additional air-dried and unstained cytologic samples were stored at room temperature in the dark for a maximum of one week. Thereafter, these slides were fixed in cold acetone (4°C) for 5 min and stored at −20°C awaiting immunocytochemistry. A proliferation of histiocytic origin was suspected based on cytomorphology, and absence of cytoplasmic granules and etiologic agents. In addition, excisional biopsies of skin nodules were performed in all cats. Tissue samples were fixed in 10% neutral-buffered formalin, processed routinely, and embedded in paraffin wax. Sections of 5 μm were stained with routine hematoxylin and eosin. A postmortem examination was performed in 4 cats (cases 1–3, and 5), and samples from skin and major internal organs were collected for microscopic examination. In one cat (case 5), cytology of the skin lesions was repeated at necropsy.

Immunocytochemistry

Immunocytochemistry was performed with a panel of primary antibodies listed in Table 1. Endogenous peroxidase was quenched with 0.3% hydrogen peroxide (Merck) and 0.1% sodium azide (Merck) in Tris buffer (Sigma-Aldrich, St. Louis, MO, USA) (0.1-M solution, pH 7.5) for 30 min and rinsed in 3 changes of Tris buffer for 5 min each. Incubation with primary antibodies was performed for one hour at room temperature in Tris buffer containing 10% inactivated serum. Providers, clones, and specific working dilutions of primary antibodies are listed in Table 1. Omission of the primary antibody or application of an isotype-matched, non–cross-reactive antibody (anti-human CD8, clone C8/144B; Dako, Glostrup, Denmark) was used as a negative control in each run. After rinsing 3 times for 5 min each with Tris buffer, secondary anti-mouse IgG biotinylated antibody (Vectastain, Burlingame, CA, USA) was applied (1:200 dilution) for 30 min. After

Table 1. List of monoclonal antibodies utilized for immunocytochemistry on cytologic smears from skin lesions in 5 cats with feline progressive histiocytosis.

Antigen	Clone	Species Specificity	Source	Dilution
CD1a	Fe1.5F4	Feline	LABL*	1:10
CD1c	Fe5.5C1	Feline	LABL*	1:10
CD3ε	A0452	Human§	Dako†	1:10
CD11b	Ca16.3E10	Canine§	LABL*	1:10
CD18	Fe3.9F2	Feline	LABL*	1:10
CD21	Ca2.1D6	Canine§	LABL*	1:10
MHC II	42.3	Feline	LABL*	1:10
E-Cadherin	4A2C7	Human§	Zymed‡	1:50

*Leukocyte Antigen Biology Laboratory, Peter F. Moore, University of California, Davis.
†Dako, Glostrup, Denmark.
‡Zymed Laboratories, San Francisco, CA.
§Cross-reactive with feline tissues.

3–5 min washes with Tris buffer, slides were incubated with the Avidin-Biotin enzyme Complex (ABC kit; Vectastain) for 30 min. The Amino-9-ethyl-carbazole (AEC) substrate kit (Dako) was used as chromogen. Finally, the slides were counterstained with Mayer's hematoxylin for 3 min, and cover-slipped with an aqueous mounting media (Glicerine; Sigma-Aldrich).

Immunohistochemistry

Immunohistochemical analysis for E-cadherin expression was performed on 5-μm formalin-fixed and paraffin-embedded histologic sections mounted on polylysine coated slides. Samples were deparaffinized, rehydrated, and then treated with 0.05% Extran (Merck) in distilled water for 10 min in a microwave oven at 900 W for antigen retrieval. Slides were cooled in phosphate-buffered saline (PBS; Sigma-Aldrich) buffer for 20 min at room temperature, washed in distilled water, and rinsed twice in PBS for 5 min each. Endogenous peroxidase was quenched by treatment with 0.3% hydrogen peroxide in methanol (Merck) for 10 min. Sections were then incubated in a humid chamber for 20 min with normal rabbit serum (Dako) diluted 1:5 in bovine serum albumin (Sigma-Aldrich). Excess serum was removed and sections were incubated with monoclonal mouse anti-human E-cadherin antibody (Table 1) diluted 1:50, overnight at 4°C. Finally, the slides were incubated with a 1:200 dilution of biotin-labeled rabbit anti-mouse secondary antibody (Dako) for 30 min. Diaminobenzidine (Dako) was applied as chromogen for 7 min, and samples were counterstained with Mayer's hematoxylin for one min.

Transmission electron microscopy

In one case, fresh tissue of cutaneous nodules was available for transmission electron microscopy (TEM). Samples of the mass were fixed in 2.5% glutaraldehyde, postfixed in 1% Osmium tretraoxide (OsO4), and embedded in epoxy resin. Electron micrographs were taken on ultrathin sections using a Zeiss EM900 electron microscope (Zeiss, Oberchoken, Germany).

Results

Clinical data

Cytologic samples were obtained from 5 Domestic Shorthaired cats, including 4 neutered females and one castrated male. Age ranged from 7 to 12 years (mean 10.8). Lesions were initially limited to the skin in all cats. Clinical presentation was characterized by variably sized multiple nodules frequently coalescing into larger plaques. The lesions were firm, alopecic, and pruritic in one cat (cat 3). At first clinical presentation, nodules were more commonly located on the head (5/5) (Figure 1A), on the limbs (4/5) and trunk (4/5), and on the digits (Figure 1B) and tail (2/5). Ulceration was present in larger and older lesions (Figures 1A,B).

Cytologic findings

Cytologic cellularity of samples varied from high to low (one cat); however, in this latter cat, highly cellular scrapings were obtained for immunocytochemistry from the cutaneous biopsies prior to formalin fixation. Main cytologic features consisted of a prevalent population of round to polygonal to rarely spindle cells with indistinct cell borders, and a moderate to abundant amount of light blue to clear homogeneous, lightly granular cytoplasm (Figure 2A,B). Large cohesive cell groups were present in 2 cats. Cytoplasmic vacuoles were present only in one case (Figure 2C). Nuclei were oval to reniform and occasionally indented, in a paracentral to peripheral location, with finely clumped to reticular chromatin and a rarely visible single round central nucleolus. Cytologic atypia was present in lesions of 4/5 cats mostly characterized by mild-to-moderate anisocytosis and anisokaryosis. Rare binucleated cells and multinucleated giant cells were present in samples from 2 cats (Figures 2A,B). Mitoses were infrequent to absent. Phagocytic activity was not observed in neoplastic cells. The main cell population was associated with a low number of small mature lymphocytes in 4 cats, nondegenerate neutrophils in 3

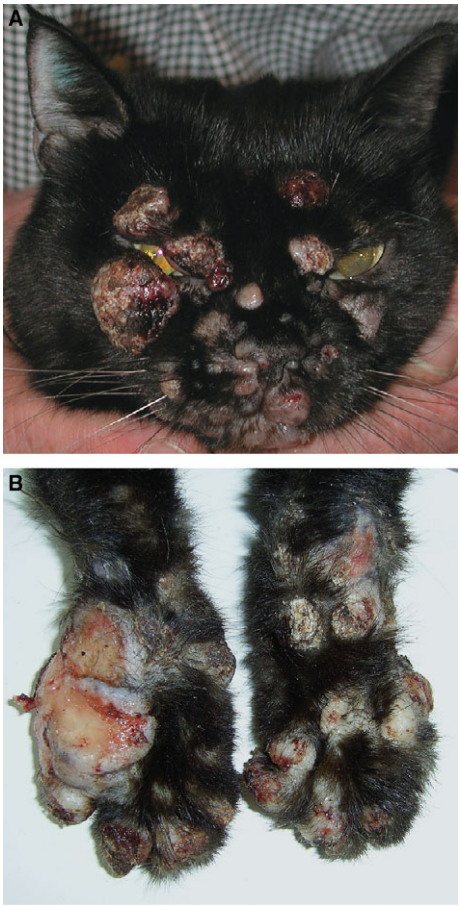

Figure 1. (**A, B**) Clinical presentation of Feline Progressive Histiocytosis in 5 cats (**A**) Multiple alopecic and ulcerated cutaneous nodules causing severe facial distortion (cat 1). (**B**) Severe deformation of digits and carpal regions by multiple coalescing nodules (cat 5).

cats (Figure 2C), and mast cells in 3 specimens. No plasma cells were observed.

Histopathologic findings

In histopathology, lesions were characterized by a dense and diffuse dermal infiltrate, nonencapsulated and poorly demarcated, composed of a monomorphic round cell population, extending from the superficial dermis into the subcutis (Figure 3A). The overlying epidermis was found either intact or ulcerated, and epitheliotropism was detected in 2 cases (cats 2 and 3, Figure 3C), with single cells or small cellular aggregates in the epidermis. Neoplastic cells were large (25–40 μm), round to polygonal, with a low to intermediate nuclear to cytoplasmic ratio, and a lightly eosinophilic cytoplasm. Occasionally, neoplastic cells exhibited numerous clear cytoplasmic vacuoles with variably distinct margins. Nuclei were round to oval to reniform, 15–20 μm in diameter, and paracentral, with finely granular to reticular chromatin and frequently a small and inconspicuous single nucleolus. Binucleated cells were occasionally present in sections from all cats, and atypical multinucleated cells were prominent in 2 cats (cats 2 and 3, Figure 3B). Additional atypical features included anisocytosis and anisokaryosis (Figure 3B), which were more prominent in sections of 3 cats (cats 1, 3, and 5). Mitotic figures ranged from 0 to 3 per high power field in all cases. In association with the main cell population, 5–30% of small mature lymphocytes (5/5 cats) and lesser numbers of neutrophils (2/5 cats) were present. Mast cells represented approximately 20% of the infiltrating cell population in cat number 3.

Immunocytochemistry

In 3/5 cats (cats 1, 2, and 3), it was not possible to apply the entire antibody panel due to the limited number of specimens. Priority was given to CD1a, CD11b, CD18, and MHC class II to confirm a macrophage or DC lineage. In cytologic samples, neoplastic cells expressed CD1a (Figure 2D), CD18 and MHCII in all cases. CD11b was positive in 4/4 cats (not performed in cat 2 due to the lack of cytologic specimen), and CD1c was positive in 2/2 cats (not performed in the other cats due to the lack of cytologic specimens). Expression of CD3 (performed in 3/5 cats) identified infiltrating small mature lymphocytes as T cells. There were no CD21-positive cells in the 2 cats where additional slides were available. CD3 and CD21 expression were not detected in the neoplastic cell population.

In skin biopsies, E-cadherin expression was observed in 4 cats (Figure 3D), while in cat 5, neoplastic cells were E-cadherin-negative.

Transmission electron microscopy

Main ultrastructural features in the lesions of cat 3 where TEM was performed consisted of cytoplasmic projections and deep invaginations of the cytoplasmic membrane, the presence of coated vesicles in the cytoplasm, pleomorphic inclusions, and a moderately complex nuclear membrane, characteristics that are all related with histiocytic cells of dendritic origin. However, no Birbeck granules were detected.

Outcome and necropsy findings

One cat (cat 4) was lost to follow-up. The other cats survived 4 months (cat 1), 6 months (cat 5), and

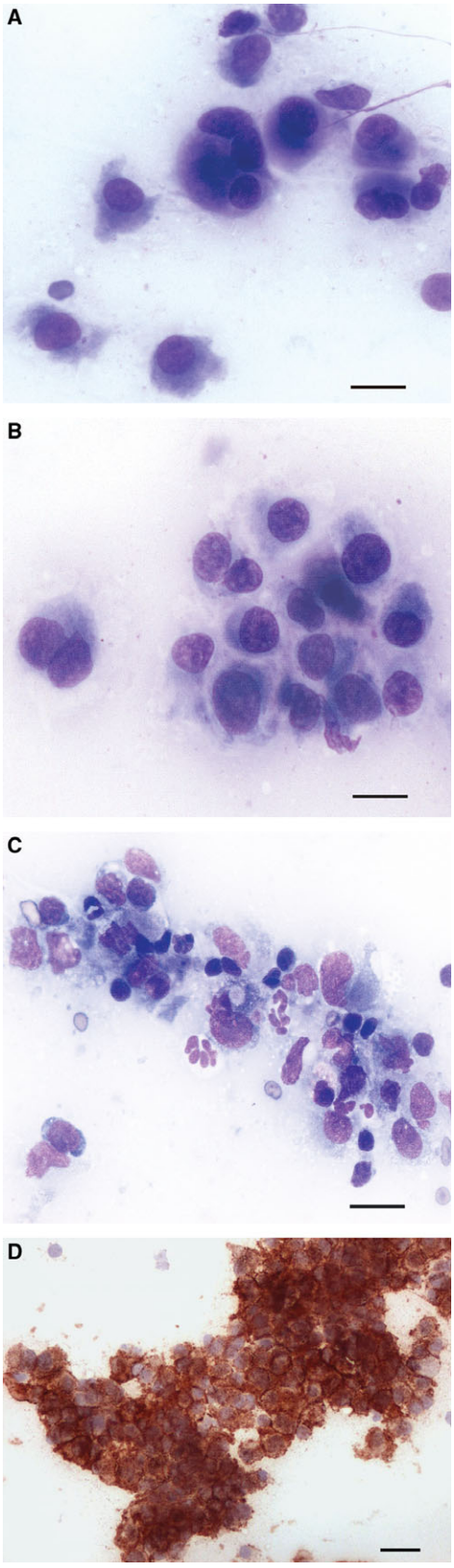

Figure 2. (**A**–**D**) Cytologic features of Feline Progressive Histiocytosis in 5 cats. (**A**) Fine-needle aspirate of a facial nodule characterized by the presence of round to spindle cells with abundant homogeneous cytoplasm and oval to indented nuclei (cat 2). One binucleated cell and one trinucleated giant cell are present. May–Grünwald–Giemsa. Bar = 12 μm. (**B**) Fine-needle aspirate of a truncal nodule characterized by discrete round to polygonal to fusiform neoplastic cells characterized by mild anysocytosis and anysokariosis (cat 2). Two binucleated cells are evident. May–Grünwald–Giemsa. Bar = 15 μm. (**C**) Fine-needle aspirate of a facial nodule (cat 5). Histiocytic cells with occasional vacuoles are admixed with nondegenerated neutrophils and small mature lymphocytes. May–Grünwald–Giemsa. Bar = 24 μm. (**D**) Large aggregate of cohesive cells demonstrating intense cytoplasmic positivity for CD1a (cat 2). Immunocytochemstry, Amino-9-ethyl-carbazole chromogen, Mayer's hematoxylin counterstain. Bar = 30 μm.

9 months (cat 2 and 3). Cats were euthanized due to progressive disease, and full necropsies were performed (cats 1, 2, 3, and 5). Cutaneous lesions had progressed in all cats, and multiple, often ulcerated nodules were present on the head (4/4), limbs (4/4), trunk (3/4), and tail (1/4). Histologically, the organs most frequently involved were the lymph nodes (3/4), spleen (1/4), liver (1/4), lungs (1/4), pancreas (1/4), and kidney (1/4). Tumor cells were not detected in the bone marrow in 3 cats, while one cat was not evaluated (cat 2). In one cat, lesions were limited only to the skin (cat 3).

Neoplastic cells invading lymph nodes and internal organs resembled the cells observed in the skin biopsies, but with increased atypical features compared with the initial specimens. Moderate to marked anisocytosis and anisokaryosis were consistent, and atypical mitoses were observed.

Discussion

FPH is a rare histiocytic disease that has been clinically and pathologically characterized, but its cytologic features have not been fully elucidated.[11] In this study, the cytologic and immunocytochemical features that allowed a diagnosis of FPH in 5 cats were identified. The cytology characterized by single to loosely cohesive histiocytic cells, together with scattered inflammatory cells, and the dendritic cell immunophenotype confirmed the diagnosis of FPH without the need of a tissue biopsy, although the diagnosis was further supported by histopathology and necropsy findings.

Early clinical manifestations of FPH are single to multiple cutaneous papules and nodules often localized on the head and extremities. As lesions progress,

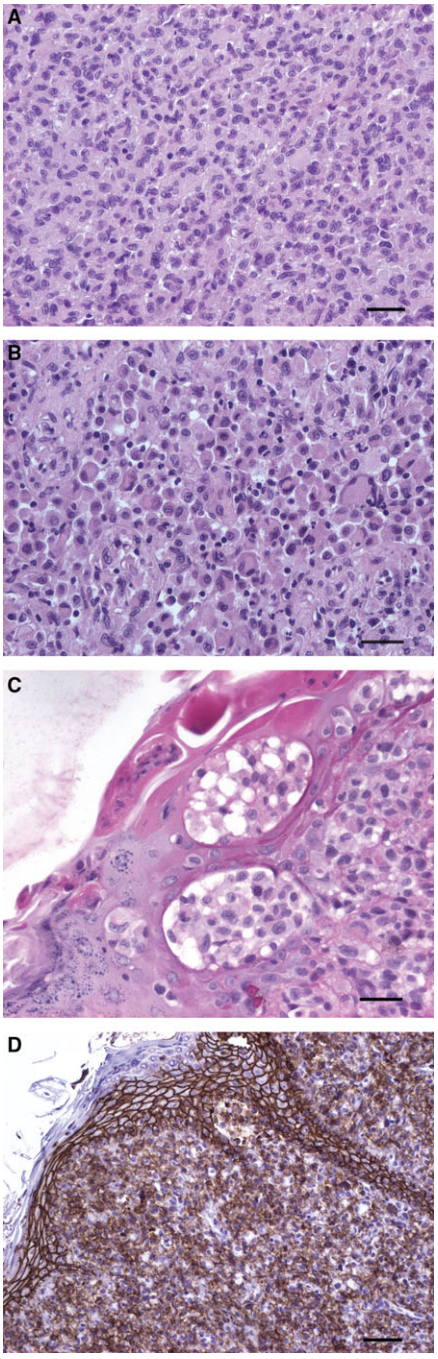

Figure 3. (**A**–**D**). Histopathologic features of Feline Progressive Histiocytosis in skin biopsies of 5 cats. (**A**) Monomorphic round cell population with indistinct cell borders and reniform nuclei substituting the dermis (cat 4). H&E, Bar = 60 μm. (**B**) Pleomorphic cell population effacing the dermis characterized by anysocytosis, anysokaryosis, binucleated and multinucleated giant cells (cat 3). H&E. Bar = 56 μm. (**C**) Presence of prominent intraepidermal aggregates of histiocytic neoplastic cells (cat 3). H&E. Bar = 48 μm. (**D**) E-cadherin expression by keratinocytes of the lower epidermis and by neoplastic cells infiltrating the dermis (cat 3). Immunohistochemistry, Amino-9-ethyl-carbazole chromogen, Mayer's hematoxylin counterstain. Bar = 100 μm.

they tend to ulcerate and increase in number and size, spreading to other cutaneous regions of the body. Late-stage FPH evolves into a disseminated histiocytic neoplastic disorder with development of metastases to lymph nodes and various internal organs.[11]

Our cases were characterized by distinct cytologic features that supported the diagnosis of FPH, including occasional cohesion, mild-to-moderate atypia, lack of phagocytosis, few cytoplasmic vacuoles, lack of degeneration of neutrophils, and lack of microorganisms. Cytologic atypia increased as lesions progressed, paralleling previous histopathologic descriptions.[11] Concurrently, cytology aided in the exclusion of several inflammatory and neoplastic disorders. Although cytology was considered highly suggestive of FPH, it was not diagnostic per se, and immunophenotyping was necessary to confirm the diagnosis.

Clinical differential diagnoses for FPH should include mycobacterial and fungal diseases, as well as xanthomas, cutaneous lymphocytosis, and other round cell tumors. Many of these entities could be excluded based on cytomorphologic features. Granulomatous inflammation is usually characterized by reactive macrophages and multinucleated giant cells. The association with etiologic agents and possibly neutrophilic degeneration that were absent in our cases, would distinguish granulomatous inflammation from early FPH cases. Xanthomas were excluded based on the absence of cytoplasmic cholesterol crystals, erythrophagocytosis, and abundant cytoplasmic vacuolation of histiocytes.[24,25] Lesions in cutaneous lymphocytosis yield a striking predominance of small mature lymphocytes.[26] Poorly differentiated mast cell tumors can resemble histiocytes, but cytologic examination usually reveals some cytoplasmic metachromatic granules even in poorly granulated tumors. Undifferentiated plasmacytoma usually does not express CD1 and CD11b.

The markers CD3, CD18, and CD21 are frequently part of the antibody panel for the identification of leukocyte origin (CD18) and exclusion of T (CD3) or B (CD21) lymphocyte origin. High-grade lymphoma was excluded by morphology and due to the lack of expression of lymphoid markers (CD3 and CD21) by the neoplastic population. The differential diagnosis of NK-like lymphoma was excluded as a result of morphologic and phenotypical evaluation as feline NK cells usually have cytoplasmic granules, are negative for CD3, CD21, and frequently also for CD11b.[27] All 4 cases evaluated for CD3 expression revealed an inflammatory T-lymphocyte infiltrate. Previous studies have identified these lymphocytes as CD8 + cytotoxic T cells.[11]

Histiocytic sarcoma, a tumor of dendritic cell origin, cannot be differentiated from late-stage FPH based on morphology and immunophenotype as both tumoral entities express CD1. The clinical history is the main feature that will help establish a definitive diagnosis of FPH, where multiple cutaneous lesions are the first manifestation of a slowly progressive disease.[11] In contrast, FSH of macrophage origin expresses CD11b, CD18, and MHCII and is CD1-negative[21], thus immunocytochemistry or immunohistochemistry on fresh tissues allow a differential diagnosis from FPH.

Still, in most cases where cytomorphology and clinical presentation are inconclusive for a final diagnosis, immunophenotyping represents an accurate diagnostic tool to help rule out other conditions. Immunophenotype should be assessed on frozen histologic tissues or on cytologic samples, as some primary antibodies that recognize surface molecules are generally damaged by formalin fixation, such as CD1. Cytologic samples represent a useful alternative that is a practical source of fresh material, eliminating the need for liquid nitrogen and isopentane, and required infrastructure for storage of frozen biopsies. In this study, immunocytochemistry allowed a rapid assessment of the cell phenotype within 4–5 h. The only limitation is the need for several air-dried cytologic samples with adequate cellularity. When aspiration techniques yield specimens with inadequate cellularity, scrapings from biopsies should be acquired for adequate immunophenotyping. Therefore, the communication between clinical pathologists and clinicians is important to obtain a sufficient number of quality slides.

In cases where the number of slides is limited, the selection of the markers is pivotal to obtain a minimal data base. Histiocytic proliferations are classified as macrophage or DC in origin, according to the immunophenotypic profile.[1] Macrophages express CD11b, CD14, and MHC II, while a DC origin is identified by expression of CD1, CD11b, and CD11c[11,28], and expression of 2–3 fold higher levels of MHC I and II than macrophages.[29] Antibodies for CD11c are not available for feline tissues.[11]

In our cases, CD11b, CD18, and MHCII expression was consistent with a histiocytic proliferation, whereas CD1 expression supported a DC origin.[11] A DC phenotype together with features of epitheliotropism has been considered indicative of a possible Langerhans cell origin.[11] DC subtypes (Langerhans cells and interstitial DC) differ in ontogeny, location, and phenotype. LC reside in the epidermis and mucosal epithelial linings. They express CD1, CD11c, MHCII, and E-cadherin, and possess Birbeck granules, the electron-microscopic hallmark of LC. Birbeck granules have not been identified in dogs, but have been reported in several species including cats.[12] In contrast, interstitial DC are located in the dermis, mainly in the nonlymphoid perivascular tissue. They lack Birbeck granules and do not express E-cadherin, but coexpress Thy-1 (CD90) and, if activated, CD4.[11]

E-cadherin is a calcium-dependent adhesion molecule that has long been considered a useful marker for LC differentiation in canine cutaneous histiocytoma.[30–32] Conversely, a recent study has demonstrated that E-cadherin expression is not limited to histiocytoma, but may be found in several canine tumors including mast cell tumors, plasmacytomas, cutaneous HS, and epitheliotropic lymphomas.[33] In a study describing FPH[11], E-cadherin was reported to be only occasionally expressed and did not correlate with epitheliotropism of tumor cells. An LC origin of FPH was initially hypothesized based on the epitheliotropic behavior, but the lack of consistent E-cadherin staining made an LC origin unlikely.[11] In our study, 4 cases, including 2 epitheliotropic tumors, were E-cadherin-positive. So, similar to previous observations[11], E-cadherin staining was not exclusive of epitheliotropic lesions. Additionally, following their activation in the epidermis by the encounter with antigens, LC downregulate their expression of E-cadherin as they migrate to the dermis. Hence, LC origin could not be confirmed nor completely ruled out, because lesional histiocytes may lose E-cadherin expression.[11]

Birbeck granules were not identified in the only case where tissue was available for ultrastructural evaluation in this study. This case expressed E-cadherin and was characterized by multifocal epitheliotropism on histology. Therefore, within this case series and similar to other reports[11] findings seem more compatible with an interstitial DC origin. As suggested in another study[11], a possible FPH origin from interstitial DC could be confirmed by electron-microscopic evaluation of more cases, or by studies on the expression of CD90 (not available for feline tissues) and of Langerin (CD207). Langerin is a valuable marker of LCs that is associated with Birbeck granule formation[34,35], and has been recently demonstrated in feline esophageal LC.[36]

In summary, our cases closely resembled the clinical and pathologic features reported previously for feline FPH cases.[11] In this report, we conclude that cytomorphology together with the clinical presentation of a slowly progressive nodular skin disease is highly suggestive of FPH, and a timely confirmation can be obtained specifically by demonstrating CD1 expression on cytologic preparations.

Preservation of an adequate number of unstained air-dried and acetone-fixed cytologic specimens is recommended to provide adequate material for immunocytochemistry without the need to collect frozen tissue specimens. Our diagnostic approach proved to be feasible and rapid, allowing a prompt management of patients, and a timely provision of the correct prognosis that is generally poor.[11]

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