



Original contribution

Langerin staining identifies most littoral cell angiomias but not most other splenic angiomatous lesions[☆]



William Selove MD^a, Jennifer Picarsic MD^b, Steven H. Swerdlow MD^{c,*}

^aDivision of Hematopathology, UPMC Presbyterian, Pittsburgh, PA 15213, USA

^bDepartment of Pathology, University of Pittsburgh School of Medicine, UPMC Children's Hospital of Pittsburgh, Pittsburgh, PA 15224, USA

^cUniversity of Pittsburgh School of Medicine, UPMC Presbyterian, Pittsburgh, PA 15213, USA

Received 24 July 2018; accepted 10 August 2018

Keywords:

Langerin;
Littoral cell angiomias;
Spleen;
Hemangioma;
Cyclin D1;
Lymph node sinus lining cells

Summary Although littoral cell angiomias (LCAs) are phenotypically well characterized, the antibodies used to support the diagnosis identify many other cells in the normal spleen, and some may be found in other angiomatous lesions. Based on a langerin/CD207+ LCA index case, langerin and other selected immunohistochemical staining was performed on 10 LCAs, 20 other splenic angiomatous lesions, and 7 reactive lymph nodes to further investigate the role of langerin as a diagnostic tool. Ninety percent (9/10) of LCAs were langerin positive, whereas only 1 (5%) of 20 other splenic vascular lesions was partially positive ($P < .00001$). All LCAs were CD1a-, CD68+, CD34-, and CD8-; 20% were S100+, 70% CD21+, and 90% cyclin D1+. Ultrastructural studies of one LCA did not show Birbeck-type granules in definite lining cells. Sinus lining cells in 7 of 7 reactive lymph nodes showed partial langerin positivity, and 4 of 4 showed partial cyclin D1 positivity. In conclusion, langerin staining is an easily interpreted and highly sensitive and specific (sensitivity [0.90], specificity [0.95]) ancillary study to help distinguish LCA from other vascular tumors of the spleen. Whether this represents cross-reactivity or true CD207 expression is uncertain, as other immunohistochemical and ultrastructural studies do not support a Langerhans cell origin. The cyclin D1 staining seen in most LCA would be consistent with their expression of other selected vascular and histiocytic markers. The similar staining pattern in some lymph node sinus lining cells suggests a possible similar cell of origin, although LCA of lymph nodes is not described.

© 2018 Elsevier Inc. All rights reserved.

1. Introduction

Littoral cell angiomias (LCAs) are a histologically and phenotypically distinctive splenic vascular neoplasm thought to be derived from red pulp sinus lining cells, which are also known as littoral cells [1,2]. LCAs need to be distinguished from other splenic angiomatous lesions such

[☆] Disclosures: None of the authors have a conflict of interest. No external sources of support.

* Corresponding author at: UPMC Presbyterian, Hill Building, Room 359, 3477 Euler Way, Pittsburgh, PA 15213.

E-mail addresses: billselove@gmail.com (W. Selove), picarsicj@upmc.edu (J. Picarsic), swerdlowsh@upmc.edu (S. H. Swerdlow).

as hemangiomas, angiosarcomas, and hamartomas. LCAs are immunophenotypically characterized by coexpression of both histiocyte-associated markers such as CD68 [1], CD163 [2,3], and lysozyme [4] and a subset of endothelial markers such as

CD31 and von Willebrand factor (vWF) [1]. Positivity for CD21 [5] and negativity for CD8 [2,6] and CD34 [2,6] are also characteristic. The diagnosis of LCA sometimes may still be challenging, in part because many of the positive immunohistochemical markers are expressed in subsets of cells in normal spleens, some are also expressed by other types of angiomatous lesions, and portions of normal spleen may be present within LCA further complicating interpretation. As part of the evaluation of histiocytic/dendritic cells in association with a presumptive LCA, we unexpectedly found expression of langerin (CD207) in the lesional lining cells, a previously unreported finding. Langerin is a C-type lectin with physiologic expression limited predominantly to Langerhans cells in the epidermis and stratified mucosal tissue [7] and to scattered positive dendritic cells in the dermis [8,9], spleen, and thymus. Positivity is used largely to help support the diagnosis of Langerhans cell neoplasms.

To assess the potential diagnostic utility of langerin in the identification of LCA, determine whether other markers of Langerhans cells would also be present, and correlate the findings with other histiocytic/dendritic markers, we investigated a series of LCAs and other splenic angiomatous lesions using a panel of antibodies that help identify varied histiocytic, dendritic, and vascular neoplasms. Furthermore, based on a previous limited report of langerin staining in the sinus lining cells of reactive lymph nodes [10], we also examined the pattern of langerin staining in lymph node biopsies. The expression of cyclin D1, which identifies subsets of histiocytes and endothelial cells, was also investigated to further aid in the comparison of LCA and lymph node sinus cells.

2. Materials and methods

The study was approved by the University of Pittsburgh Institutional Review Board.

2.1. Case selection

The existing histologic sections and immunohistologic stains from 30 splenic angiomatous lesions from 29 individuals (28 splenectomies and 1 core biopsy) received from 2003 to 2018 were reviewed and the diagnoses confirmed. Seven reactive lymph nodes from 7 individuals were also identified.

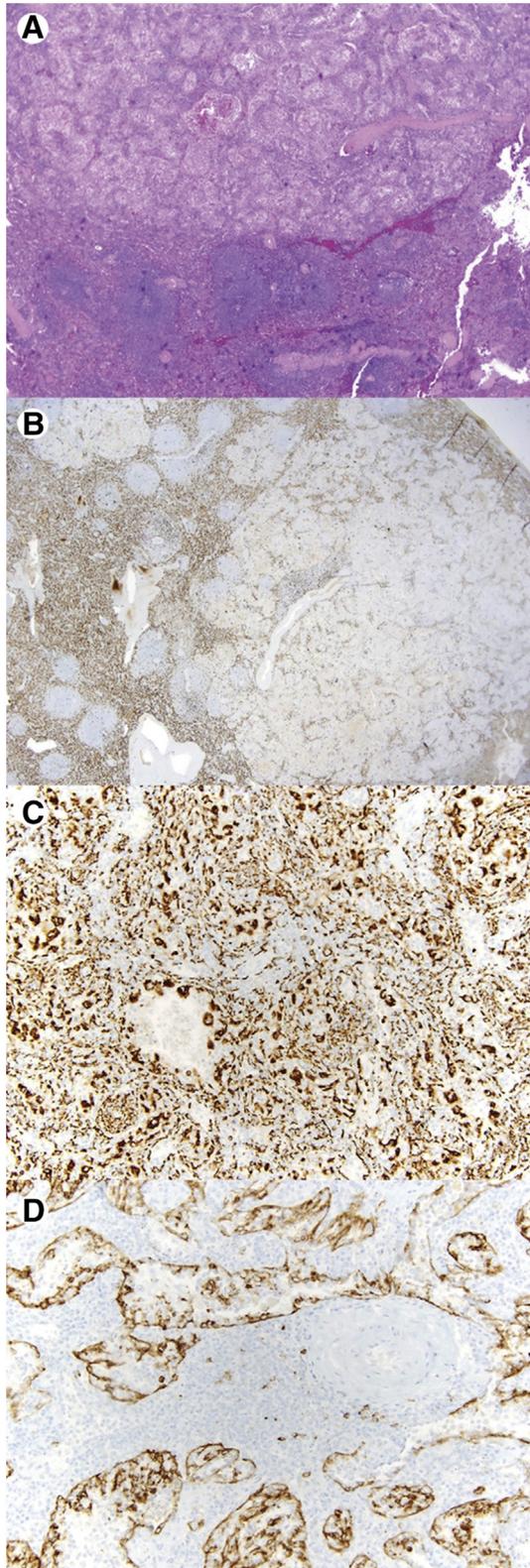


Fig. 1 LCA. A, The LCA is in the upper portion with uninvolved spleen below (hematoxylin and eosin, original magnification $\times 4$). B, CD8 stains the normal sinus lining cells, but the lesional lining cells are negative ($\times 2$). C, CD68 is positive in the lesional lining cells and in background histiocytes ($\times 20$). D, Langerin highlights the lesional sinus lining cells with a distinct appearance unlike that of Langerhans cell histiocytosis. Otherwise, there are only a few positive scattered dendritic cells ($\times 20$).

Table LCA immunohistochemistry

	Langerin (%)	S100 (%)	CD1a (%)	CD34 (%)	CD8 (%)	CD68 (%)	CD21 (%)	CD35 ^a (%)	Cyclin D1 (%)
Positive	90	20	0	0	0	100	70	66	90
Diffuse	40 ^b	0	0	0	0	60	0	22	0
Partial	50	20	0	0	0	40	70	44	88

^a Only 9 of 10 cases were stained.

^b One of the multiple lesions had partial staining.

2.2. Immunohistochemical studies

Paraffin blocks were retrieved in all cases and stained for langerin (CD207) (12D6, predilute; Cell Marque, Rocklin, CA). All LCA cases were also stained with antibodies for CD1a (EP3622, predilute; Ventana, Tuscon, AZ), S100 (dilution 1:500; Dako, Santa Clara, CA), cyclin D1 (SP4, dilution 1:100; Thermo Scientific, Pittsburgh, PA), CD34 (QBEnd/10, predilute; Ventana), CD8 (C8/144B, dilution 1:40; Dako), CD68 (KP-1, predilute; Ventana), CD21 (1F8, predilute; Ventana), and 9 of 10 cases stained for CD35 (RLB25, predilute; Cell Marque). All non-LCA cases were stained for CD34 and CD8. Selected other markers were used to better characterize lesions, when necessary. Other antibodies used included

vWF (dilution 1:500; Dako), CD31 (JC70A, dilution 1:40; Dako), and D2-40 (D2-40, predilute; Ventana). The 7 reactive lymph nodes were stained for langerin and 4 stained for cyclin D1. All stains were performed using a Ventana Bench Mark XT (Ventana) automated immunostainer. Stains were considered extensively positive if greater than 75% of cells of interest showed expression and partially positive if at least 5% positive cells were present, which were at least focally contiguous.

2.3. Electron microscopy

A small portion of tissue was deparaffinized from a paraffin block from 1 LCA stepwise using xylene, decreasing concentrations of alcohol, then cacodylate buffer. The tissue was then

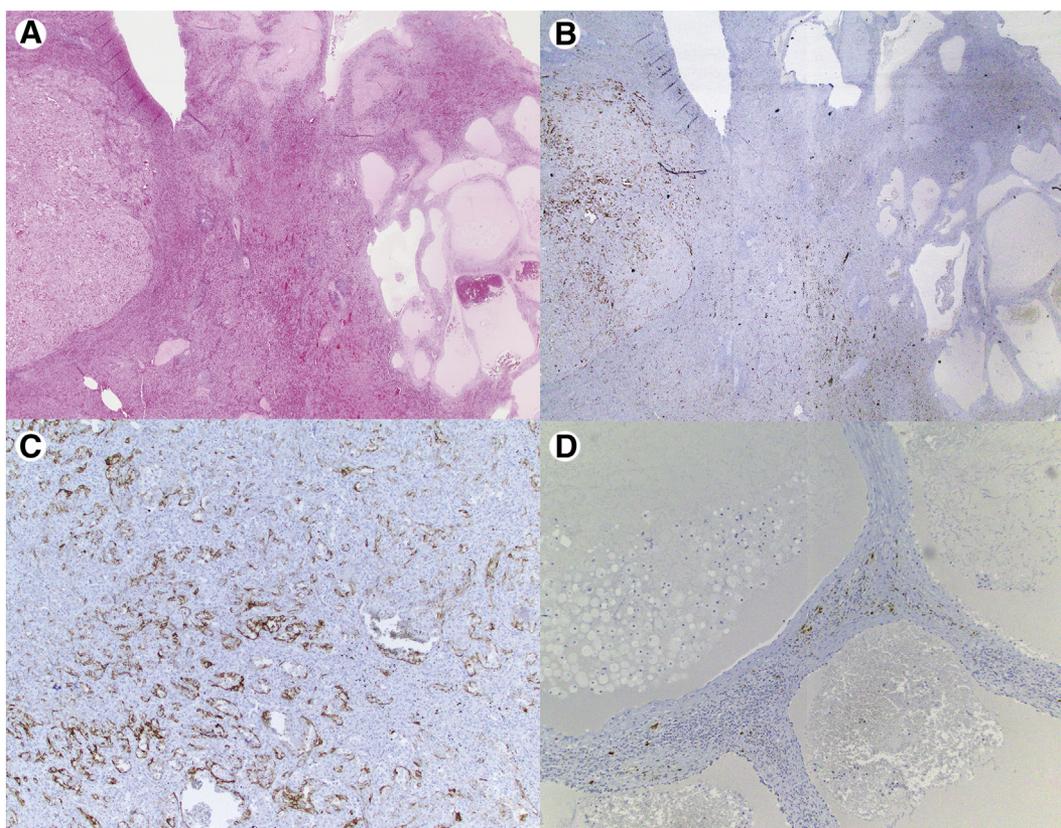


Fig. 2 LCA and lymphangioma. A, Note the LCA on the left and a lymphangioma on the right (hematoxylin and eosin, original magnification $\times 2$). B, The LCA shows strong staining for langerin, while the lymphangioma is negative ($\times 2$). The apparent staining in the area between the lesions is hemosiderin. C, At higher magnification, the langerin staining of the lining cells is seen ($\times 10$). D, The lymphangioma, however, is negative for langerin ($\times 10$).

fixed in Karnovsky fixative, postfixed in 1% osmium tetroxide, embedded in Epon-Araldite, cut at 900 Å, and analyzed on Tecnai Spirit Electron Microscope (Hillsboro, OR) looking for Birbeck granules in lesional cells.

3. Results

Patients ranged in age from 18 to 91 years (median, 65 years), with 12 men and 17 women. The angiomatous lesions included 10 LCAs, 14 hemangiomas, 2 lymphangiomas, 1

sclerosing angiomatoid nodular transformation, 1 hamartoma, and 2 angiomatous lesions, not further classifiable. The patients with LCA had undergone splenectomy for immune thrombocytopenia (2 cases), lesions found on imaging (5 cases), splenomegaly with pancytopenia (1 case), splenomegaly with associated splenic marginal zone lymphoma (1 case), and involvement by perinephric abscess (1 case, the spleen also associated with lymphangioma). Patients with other angiomatous lesions had splenectomies/biopsies performed for lesions found on imaging (9 cases), splenomegaly with or without cytopenias (5 cases), trauma/bleeding (4 cases), and spleen mottling seen intraoperatively during malignant colon

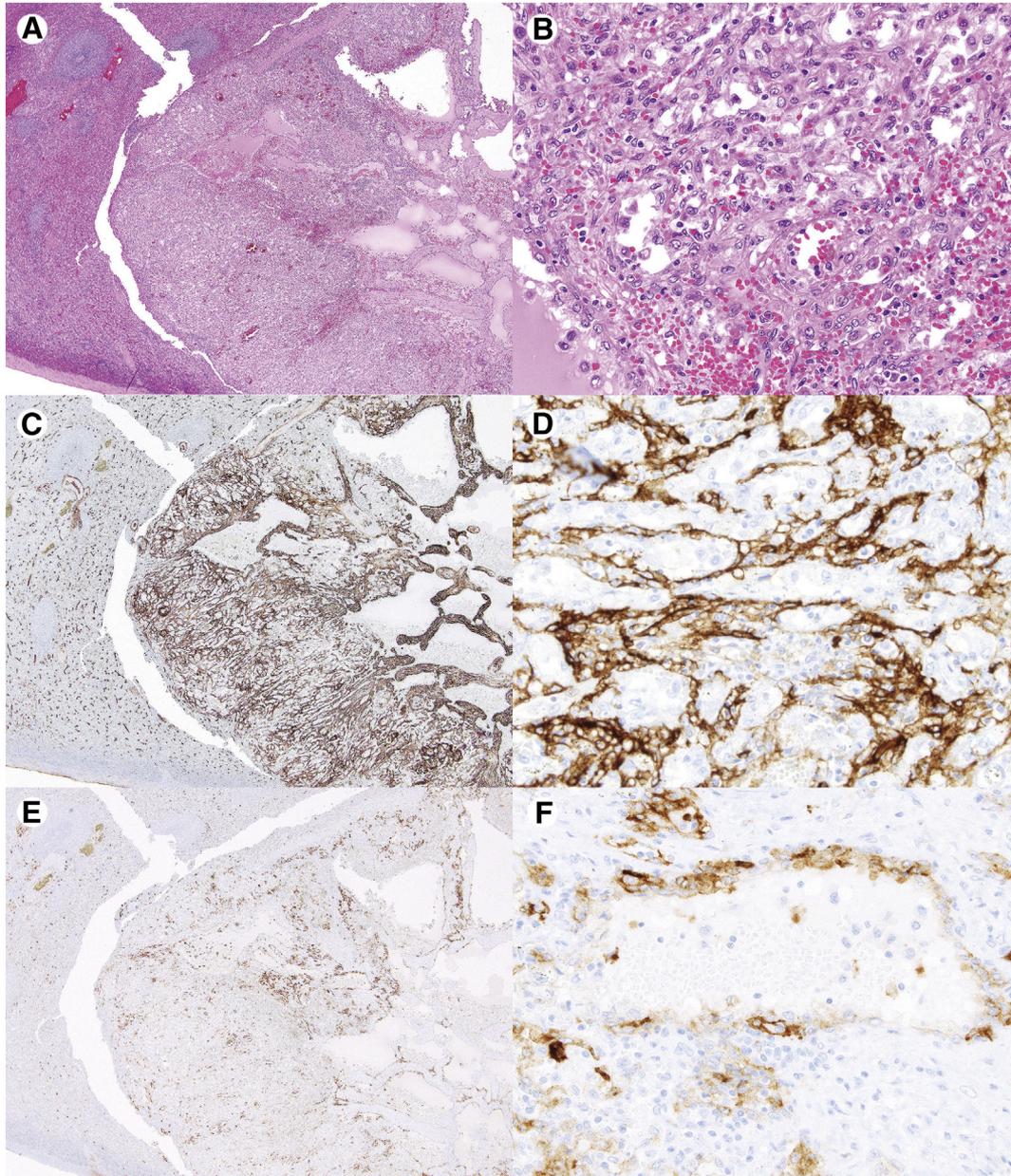


Fig. 3 LCA. A and B, LCA at low and higher magnification (hematoxylin and eosin, original magnifications $\times 4$ and $\times 40$). C, CD34 stains numerous endothelial cells ($\times 4$). D, However, at higher magnification, the actual lesional lining cells are negative ($\times 40$). Langerin highlights the lesion at low magnification (E, $\times 4$), and at higher magnification, it clearly stains lesional lining cells (F, $\times 40$).

resection (1 case). One of the non-LCA angiomatous lesions was associated with chronic lymphocytic leukemia, and 6 were associated with synchronous solid malignancies (4 renal cell carcinoma, 1 invasive ductal carcinoma of the breast, and 1 colon adenocarcinoma).

3.1. Langerin and other immunohistochemical findings

Nine of 10 LCA cases had diffuse (4) or partial (5) langerin-positive lining cells (Fig. 1, Table), whereas only 1 of 20 other angiomatous lesions (a somewhat unusual hemangioma; data not shown) had partial langerin staining. Very few scattered langerin-positive cells were present in the background red pulp of most cases. One spleen showed both a langerin-positive LCA and a langerin-negative lymphangioma (Fig. 2). LCAs were all positive for CD68 and negative for CD34, CD8, and CD1a. However, interpretation of the langerin was often easier than with CD68, CD34, and CD8 because of staining of non-lesional cells with these latter markers, sometimes even within the LCA (Fig. 3). Cyclin D1 was positive in 90%, CD21 in 70%, and S100 in 20% of the LCAs.

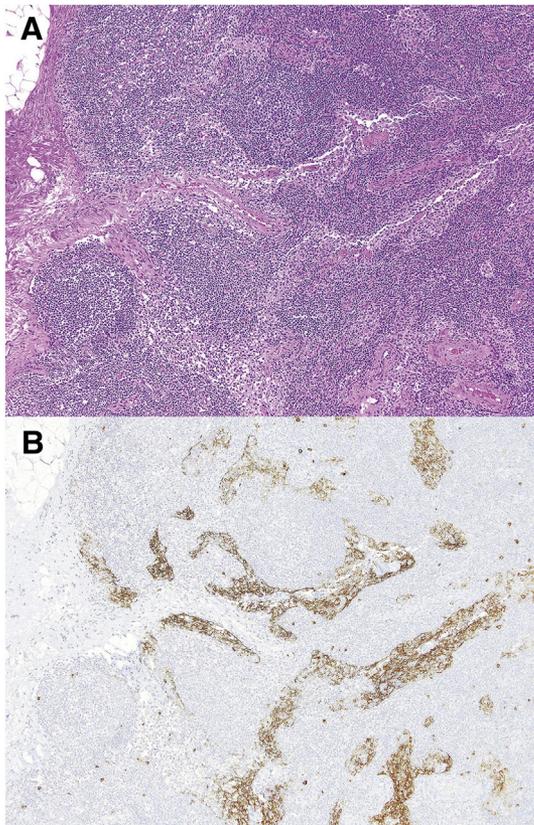


Fig. 4 Reactive lymph node. A, The normal architecture of the lymph node is intact with open sinuses (hematoxylin and eosin, original magnification $\times 10$). B, The langerin stain highlights sinus lining cells and single scattered paracortical cells consistent with Langerhans cells ($\times 10$).

All 7 reactive lymph nodes demonstrated partial langerin-positive sinus lining cells, and all 4 lymph nodes tested had partial cyclin D1-positive sinus lining cells (Fig. 4).

3.2. Ultrastructural findings

Electron microscopy performed on 1 LCA case showed rare cytoplasmic Birbeck-type granules in a single cell; however, it was not clearly one of the LCA lining cells. It may represent a reactive dendritic cell.

4. Discussion

LCAs are angiomatous lesions that are found exclusively in the spleen and are often variably sized and multiple. They are benign and usually discovered incidentally, but must be distinguished from other angiomatous lesions that may be more widely disseminated such as hemangiomas and hemangioendotheliomas or even overtly malignant such as angiosarcomas.

Splenic littoral cells, also known as sinus lining cells, line the red pulp splenic sinuses and have hybrid immunophenotypic features overlapping those of macrophages and endothelial cells. Ogembo et al [3] proposed a gatekeeper function of littoral cells that integrates features of endothelial, macrophage/dendritic, and smooth muscle cells. According to their model, littoral cells admit or deny red cells, leukocytes and other cells re-access back to the systemic circulation from the red pulp based on their profile of surface ligand expression.

LCAs are thought to be derived from littoral cells based on shared expression of lysozyme, HLA class II, NSE, CD31, Duffy antigen/chemokine receptor (DARC, CD234, gp-Fy), and signal regulatory protein α [3,4]. However, in contrast to normal littoral cells, LCAs are characterized by the expression of selected histiocytic (CD68, CD163) and dendritic (CD21) cell markers, whereas they lack CD8 and formin homology 2 domain containing 1 expression [3]. The cases studied here had typical CD68 positivity and CD34 and CD8 negativity. CD21 expression found here in 70% of LCA is a complement receptor found predominantly on B cells and follicular dendritic cells. It has been reported, however, to also be expressed in 10% to 84% of LCAs, and its precise significance is uncertain [5,11,12].

In addition to their distinctive morphologic appearance, LCAs are immunophenotypically distinct from other splenic vascular tumors, with a unique CD68/CD163-positive, CD21-frequently positive, CD8-negative, CD34-negative immunohistochemical profile. Nonetheless, LCA sometimes may be challenging to diagnose both because of frequent staining of nonneoplastic background elements that can even be within the LCA lesions and because of occasional partially shared phenotypic features with other types of vascular lesions. CD68 interpretation is complicated by the abundant background histiocytes typically present in the splenic red pulp. CD21 is also expressed in follicular dendritic meshworks

and subsets of B cells present in the spleen. CD8 negativity distinguishes LCA from normal splenic sinuses, but entrapment of nonneoplastic sinuses within the lesion can obscure interpretation. Although absence of CD34 expression helps distinguish LCA from many other angiomatous lesions, CD34 is also negative in splenic hamartomas and prominent CD34-positive vascular channels can be present within LCA. For all these reasons, it is of great practical value that the previously undescribed langerin expression in most LCA is a highly sensitive (0.90) and specific (0.95) immunohistochemical finding in the distinction of LCA from other angiomatous lesions of the spleen. Moreover, langerin staining, even when partial, was distinct and well defined within the lining cells, with very little staining of other cells in the spleen, making it more easily interpretable in most cases than the other markers commonly used in the diagnosis of LCA. The one non-LCA angiomatous lesion in our cohort with some langerin positivity shared some other features with LCA (vWF+, CD34 not clearly positive), although the lining cells were mostly very flattened.

Whether the langerin positivity reflects specific staining or some type of cross-reactivity with another C-type lectin or other antigen remains uncertain. The LCA cells did not show other immunophenotypic characteristics of Langerhans cells, being negative for CD1a and positive for S100 in only 2 cases, but whether there might be langerin expression in a non-Langerhans cell, although unlikely, is not established. Reported expression of S100 in LCA has been variable, and the explanation for its presence is uncertain [1,5,12]. S100 is a protein, found in interdigitating reticulum cells, Langerhans cells, and many other cell types, that performs numerous functions in macrophages and dendritic cells including important roles in cell growth, differentiation, and inflammatory response [13]. The lack of a Langerhans cell origin for the LCA is further confirmed from the limited ultrastructural studies that did not show Birbeck granules in definite lesional cells in the case reported here, and they have not been described in previous ultrastructural studies of LCA [14,15].

In addition to langerin, we report for the first time that LCA lining cells are cyclin D1 positive in many cases, similar to the staining pattern in many normal lymph node sinus lining cells. Cyclin D1 acts mainly as cell cycle regulator in a number of cell types but has also been suggested to play a key role in activation of macrophages as a mediator of interferon- γ signaling [16,17]. It is of interest that cyclin D1, like langerin, is positive in LCA but not in normal splenic littoral cells.

Given that many of the previously reported up-regulated markers specific to LCA versus normal littoral cells, such as CD163, CD21, and S100 (subset) play roles in immune mediation [3,18], it is of interest that langerin and cyclin D1 also have been associated with immune response. If langerin positivity in our study reflects true expression of langerin or some other C-type lectin or immune receptor, langerin and cyclin D1 could represent additional manifestations of immune dysregulation. Prior reports have noted an association between LCA and autoimmune disease [19,20] and malignancy [2], with a

postulated explanation being LCA-associated immune dysregulation [19].

Staining for both langerin and cyclin D1 was also present in lymph node sinus lining cells, both of which have been previously reported [10,21]. Chikwava and Jaffe [10] reported distinctive langerin positivity in lymph node sinus lining cells in reactive lymph nodes using the same 12D6 clone used here and in the absence of CD1a expression, as well as in hepatic sinusoids. This langerin staining, which can be quite pronounced, is important to recognize so that it is not confused with Langerhans cell histiocytosis, which is a sinus-based proliferation. Cyclin D1 expression has previously been described in the sinus lining cells of reactive lymph nodes [21]. Despite both LCA and normal lymph node (but not splenic) sinus lining cells being both langerin and cyclin D1 positive, whether they are related in some way to each other remains to be determined.

5. Conclusions

Although the precise explanation for the langerin staining patterns reported here remains uncertain, it is an easily interpreted, very sensitive, and specific stain for LCA in the context of angiomatous lesions in the spleen, even if there are exceptions. Together with cyclin D1, it is a finding also shared with a subset of normal lymph node sinus lining cells.

Acknowledgment

The authors thank Ms Patricia (Patty) Snyder for her expert technical assistance with the ultrastructural image analysis.

References

- [1] Falk S, Stutte HJ, Frizzera G. Littoral cell angioma. A novel splenic vascular lesion demonstrating histiocytic differentiation. *Am J Surg Pathol* 1991;15:1023-33.
- [2] Peckova K, Michal M, Hadravsky L, et al. Littoral cell angioma of the spleen: a study of 25 cases with confirmation of frequent association with visceral malignancies. *Histopathology* 2016;69:762-74.
- [3] Ogembo JG, Milner Jr DA, Mansfield KG, et al. SIRPalpha/CD172a and FHOD1 are unique markers of littoral cells, a recently evolved major cell population of red pulp of human spleen. *J Immunol* 2012;188:4496-505.
- [4] Buckley PJ, Smith MR, Braverman MF, Dickson SA. Human spleen contains phenotypic subsets of macrophages and dendritic cells that occupy discrete microanatomic locations. *Am J Pathol* 1987;128:505-20.
- [5] Arber DA, Strickler JG, Chen YY, Weiss LM. Splenic vascular tumors: a histologic, immunophenotypic, and virologic study. *Am J Surg Pathol* 1997;21:827-35.
- [6] Lee H, Maeda K. Hamartoma of the spleen. *Arch Pathol Lab Med* 2009;133:147-51.
- [7] 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;29:2695-704.
- [8] Bigley V, McGovern N, Milne P, et al. Langerin-expressing dendritic cells in human tissues are related to CD1c+ dendritic cells and distinct from Langerhans cells and CD141high XCR1+ dendritic cells. *J Leukoc Biol* 2015;97:627-34.
- [9] Braz-Silva PH, Vitale S, Butori C, et al. Specific infiltration of langerin-positive dendritic cells in EBV-infected tonsil, Hodgkin lymphoma and nasopharyngeal carcinoma. *Int J Cancer* 2011;128:2501-8.
- [10] Chikwava K, Jaffe R. Langerin (CD207) staining in normal pediatric tissues, reactive lymph nodes, and childhood histiocytic disorders. *Pediatr Dev Pathol* 2004;7:607-14.
- [11] Bi CF, Jiang LL, Li Z, Liu WP. Littoral cell angioma of spleen: a clinicopathologic study of 17 cases. *Zhonghua Bing Li Xue Za Zhi* 2007;36:239-43.
- [12] Neuhauser TS, Derringer GA, Thompson LD, et al. Splenic angiosarcoma: a clinicopathologic and immunophenotypic study of 28 cases. *Mod Pathol* 2000;13:978-87.
- [13] Donato R, Cannon BR, Sorci G, et al. Functions of S100 proteins. *Curr Mol Med* 2013;13:24-57.
- [14] Du J, Shen Q, Yin H, Zhou X, Wu B. Littoral cell angioma of the spleen: report of three cases and literature review. *Int J Clin Exp Pathol* 2015;8:8516-20.
- [15] Michal M, Skalova A, Fakan F, Koza V, Svojgrova M. Littoral cell angioma of the spleen. A case report with ultrastructural and immunohistochemical observations. *Zentralbl Pathol* 1993;139:361-5.
- [16] Dey A, Li W. Cell cycle-independent induction of D1 and D2 cyclin expression, but not cyclin-Cdk complex formation or Rb phosphorylation, by IFN γ in macrophages. *Biochim Biophys Acta* 2000;1497:135-47.
- [17] Neumeister P, Pixley FJ, Xiong Y, et al. Cyclin D1 governs adhesion and motility of macrophages. *Mol Biol Cell* 2003;14:2005-15.
- [18] Fabrick BO, van Bruggen R, Deng DM, et al. The macrophage scavenger receptor CD163 functions as an innate immune sensor for bacteria. *Blood* 2009;113:887-92.
- [19] Cordesmeyer S, Putzler M, Titze U, Paulus H, Hoffmann MW. Littoral cell angioma of the spleen in a patient with previous pulmonary sarcoidosis: a TNF-alpha related pathogenesis? *World J Surg Oncol* 2011;19(9):106. <https://doi.org/10.1186/1477-7819-9-106>.
- [20] Johansson J, Bjornsson B, Ignatova S, Sandstrom P, Ekstedt M. Littoral cell angioma in a patient with Crohn's disease. *Case Rep Gastrointest Med* 2015;2015:474969.
- [21] Abdulla Z, Turley H, Gatter K, Pezzella F. Immunohistological recognition of cyclin D1 expression by non-lymphoid cells among lymphoid neoplastic cells. *APMIS* 2014;122:183-91.