Use of PD-1, CD1a, and S-100 in Differentiating Pseudolymphomatous Folliculitis and Indolent Primary Cutaneous B-Cell Lymphomas

CONTENTS

1 ABSTRACT

Background: Pseudolymphomatous folliculitis (PLF) is a non-neoplastic lymphoid proliferation that clinically and histopathologically mimics primary cutaneous extranodal marginal zone lymphoma of mucosa-associated lymphoid tissue (MALT lymphoma). In this study we assessed the diagnostic value of three immunohistochemical markers, PD-1, CD1a, and S100.

Methods: We evaluated 25 cases of cutaneous lymphoid proliferations with established diagnoses, including 9 patients with PLF, 11 with MALT lymphoma, and 5 with cutaneous lymphoid hyperplasia. The clinical, histopathologic, and immunohistochemical characteristics were reviewed and three major characteristics assessed: 1) proportion of T cells expressing PD-1, 2) pattern of expression of CD1a by dendritic cells, and 3) pattern of expression of S100 by dendritic cells.

Results: We found PLF to have a significant increase in PD-1+ T cells compared to MALT lymphoma ($p<0.0001$). The pattern of CD1a staining is also informative: MALT lymphoma is significantly more likely to demonstrate a peripheral concentration of CD1a+ dendritic cells around lymphoid nodules than PLF ($p<0.0003$) or CLH ($p<0.05$). PLF demonstrates an interstitial distribution of CD1a+ cells more often than MALT lymphoma ($p<0.04$). S100 staining was not a helpful discriminator.

Conclusions: Histopathological factors including PD-1 and CD1a staining patterns may allow for more certainty in distinguishing PLF from MALT lymphoma.

2 GLOSSARY

ANOVA—analysis of variance

CBC—complete blood count

CD1a—cluster of differentiation 1a

CLH—cutaneous lymphoid hyperplasia

CT—computed tomography

FISH—fluorescence *in situ* hybridization

H&E—hematoxylin and eosin

IHC—Immunohistochemical

LDH—lactate dehydrogenase

MALT lymphoma—extranodal marginal zone lymphoma of mucosa associated lymphoid tissue (synonymous with pcMZL)

NCCN— National Comprehensive Cancer Network

PAS—periodic acid schiff

pcDLBCL—primary cutaneous diffuse large B-cell lymphoma, leg type

pcFCL—primary cutaneous follicle center lymphoma

pcMZL—primary cutaneous marginal zone lymphoma (synonymous with MALT lymphoma)

pcSMTCL—primary cutaneous small/medium T-cell lymphoma

PCR—polymerase chain reaction

PD-1—programmed death-1

PLF—pseudolymphomatous folliculitis

sMZL—extra- cutaneous marginal zone lymphoma *or* systemic marginal zone lymphoma

SPEP—serum protein electrophoresis

WHO-EORTC—World Health Organization-European Organization for Research and Treatment of Cancer

3 BACKGROUND

3.1 INTRODUCTION

The skin, one of the most important organs in the human immune system, plays a key role in both adaptive and innate immunity. A variety of immune cells reside in the skin, including T cells and dendritic cells; infiltrates that contain B cells, neutrophils, natural killer cells, eosinophils, monocytes, macrophages, and mast cells may occur (1). Immune dysregulation of any of these cell types may result in the develop of a neoplasm or cutaneous lymphoma (2).

Cutaneous lymphomas thus include an array of neoplasms that vary immensely in their clinical presentation, prognosis, histopathology, immunohistochemistry, and molecular biology. The World Health Organization-European Organization for Research and Treatment of Cancer (WHO-EORTC) classification system recognizes fifteen cutaneous lymphomas, divided into three major categories: T-cell lymphomas, B-cell lymphomas, and precursor neoplasms. In this thesis we will primarily be concerned with the primary cutaneous B-cell lymphomas.

Of tremendous importance in dermatopathology is differentiating actual lymphomas from dense dermal infiltrates that clinically and histologically mimic lymphoma. One rare but clinically significant lymphoma mimic, pseudolymphomatous folliculitis (PLF), can be remarkably difficult to differentiate from a particular form of indolent primary cutaneous B-cell lymphoma called primary cutaneous marginal zone lymphoma (pcMZL), also known as extranodal marginal zone lymphoma of mucosa-associated lymphoid tissue (MALT lymphoma). This entity will be referred to as MALT lymphoma throughout this thesis.

3.2 PSEUDOLYMPHOMATOUS FOLLICULITIS[1]

Pseudolymphomatous folliculitis (PLF), a variant of cutaneous lymphoid hyperplasia, classically presents on the scalp, face, or upper trunk as a solitary, violaceous 0.5-3 cm domed nodule without ulceration or scaling (3–14). These lesions are markedly indolent and typically regress spontaneously within weeks or months (15).

PLF was first described in 1986 by McNutt; this non-neoplastic lymphocytic infiltrate usually manifests histologically as a dense, patchy or diffuse dermal infiltrate of small round lymphocytes (3–6,11,15). Hair follicles in PLF are hyperplastic, branching and irregularly shaped with intra-follicular dendritic cells. Granulomas may also be present (3,6) while neutrophils and eosinophils are typically absent (15). Cytologically, the cells of PLF are benign appearing in terms of size and morphology (15). McNutt noted that although the histology can be concerning, typically the nodules of PLF regress within 3-4 weeks of biopsy. Rapid regression helps distinguish this non-neoplastic lymphoid infiltrate from other cutaneous lymphomas, which do not resolve after biopsy (15).

PLF was further defined as a unique subtype of cutaneous lymphoid hyperplasia (CLH) in 1999 by Arai *et al.* That group distinguished PLF from other variants of CLH given the presence of lymphocytic infiltration into pilosebaceous unit epithelium. This invasion causes architectural distortion, but not destruction, of the hair follicle epithelium and outer root sheath. Since that paper, numerous case reports and small series have been published, reinforcing the concept that PLF has distinct histopathologic and clinical characteristics.

[1] The text of this section contains selections from the paper: Goyal *et al*. "PD-1, S-100, and CD1a Expression in Pseudolymphomatous Folliculitis, Primary Cutaneous Marginal Zone B-cell Lymphoma (MALT lymphoma), and Cutaneous Lymphoid Hyperplasia." *Journal of Cutaneous Pathology*, Dec 11, 2014.

Although PLF was initially characterized solely based on histopathologic characteristics, the advent of immunohistochemistry has allowed further definition of PLF. In their 1999 paper, Arai *et. al.* described the infiltrate of PLF as a heterogeneous mix of CD3+ T cells and CD20+ B cells, with increased numbers of S-100 positive or CD1a positive dendritic cells within hair follicle epithelium as compared to other forms of CLH (4). The combination of histopathologic and immunohistochemical findings have become key in diagnosing PLF (3,4,7–10,12,14).

The differential diagnosis of PLF includes ruptured hair follicle or folliculitis and indolent primary cutaneous B-cell lymphomas, the most important of these lymphomas being primary cutaneous marginal zone lymphoma (MALT lymphoma). PLF can be distinguished from hair follicle rupture or folliculitis based on several characteristics. Findings present in folliculitis but absent in MALT lymphoma include abscess formation, giant cells, and keratin reaction. PLF further differs in that there is infiltration of the hair follicle epithelium by lymphocytes but no destruction of the follicular epithelium. Additionally, folliculitis or ruptured hair follicles usually do not demonstrate the hair follicle hyperplasia or branching characteristic of PLF. Differentiating PLF and MALT lymphoma can be extremely challenging, and is the subject of this investigation.

3.3 MALT LYMPHOMA[2]

Primary cutaneous marginal zone lymphoma (MALT lymphoma) is the second most common cutaneous B-cell lymphoma, accounting for nearly 25% of cutaneous B-cell lymphomas (16). The skin is the second most common site for extranodal marginal zone lymphomas overall, after the gastrointestinal tract (17). Although the median age of patients with this lymphoma is 50-53 years, patients range from 21-93 years old. This lymphoma is twice as common in men as women (18–20).

[2] The text of this section is excerpted from the book chapter: Goyal *et al.* "Primary cutaneous marginal zone lymphoma." *Atlas of Cutaneous Lymphomas: Classification and Differential Diagnosis*. Eds. Joi B. Carter, Amrita Goyal, Lyn M. Duncan. Springer, 2015 (82).

MALT lymphoma typically presents with a solitary or regional grouping of deep seated, red to violaceous indurated plaques, nodules, or tumors which may be surrounded by diffuse or annular erythema (*see* fig 16-1) (17,18,21). The lesions have a predilection for the trunk (46-60%), upper extremities (17%), face and scalp (13%) (11,18,19). Most reports are of relatively small series, leading to wide variability in the presentations described: 28-58% of patients present with a single lesion, 24-72% with multifocal regional lesions, and 0-17% with disseminated disease (18,19). Patients are otherwise asymptomatic. Although nodal dissemination and large cell transformation are possible, the vast majority of patients never experience extra-cutaneous spread of their disease (18,19,22).

Studies have offered some evidence of an association between MALT lymphoma and *Borrelia burgdorferi* in European populations, analogous to the role of *H. pylori* in the pathogenesis of gastric marginal zone lymphoma (MZL or MALT lymphoma). However, this has not been substantiated in North American or Asian studies (19). While many gastric MZLs demonstrate complete resolution with antibiotic therapy against *H. pylori*, a handful of case reports from Europe describe minimal to partial improvement of cutaneous MALT lymphomas with antibiotic treatment for *B. burgdorferi* infection (23,24).

MALT lymphoma is markedly indolent, with a very low incidence of extracutaneous spread and minimal risk of death (18,25). The overall 5-year survival rate is estimated at nearly 97% (22). The lesions of MALT lymphoma are very responsive to treatment: 93% of patients with solitary lesions and 75% of those with multifocal lesions attain a complete response to therapy (18). However, 39% and 77% of those patients, respectively, will experience relapses within 5 years (18).

The only factor with prognostic value is the number of lesions—patients with a single lesion have a higher survival rate than those with regional or disseminated disease (19,22). Poor prognosis has been reported in rare cases of large cell transformation or MALT lymphoma with head and neck involvement (21).

Although there is no standardized treatment protocol, single or localized lesions are typically treated with intralesional steroids, surgical excision and/or radiotherapy (18). Recurrent lesions tend to be outside of the irradiated area. Retrospective studies have demonstrated a response rate to systemic rituximab approaching 90% (26).

This lymphoma is characterized histopathologically by a dense, nodular, non-epidermotropic, dermal and subcutaneous lymphocytic B-cell infiltrate comprised of centrocyte-like marginal zone cells, lymphoplasmacytoid cells, and plasma cells (17–19,21). The cellular composition can range from prominent plasma cell infiltrates to a predominance of monocytoid B cells with few plasma cells. Zones of neoplastic plasma cells occur in more than two-thirds of cases (20,27). Reactive germinal centers are present in most cases, and are occasionally colonized by neoplastic cells (28).

The centrocyte-like marginal zone cells have cleaved nuclei with dispersed chromatin and pale amphophilic cytoplasm (17,21); the large amount of cytoplasm can give a monocytoid appearance (17). The reniform (bean-shaped) nuclei usually have inconspicuous nucleoli (17,29). Dutcher bodies (intranuclear PAS+ immunoglobulin pseudo-inclusions found in plasma cells) may also be present.

Findings that may confound the identification of the neoplastic B cells include a typically dense infiltrate of benign reactive CD3+ CD45RO+ T-cells (17,21). Scattered centroblasts and

immunoblasts (from disrupted follicle centers) may be seen, particularly in the setting of follicular colonization. Variable numbers of CD30+ large cells may also be present (21). Adnexal infiltration by lymphocytes may be seen; both eccrine and follicular involvement have been reported (27). Formation of lymphoepithelial lesions (groups of 3 or more marginal zone cells with destruction of epithelium) involving adnexal structures is diagnostically helpful but often absent (17,21).

The neoplastic B cells have an immunohistochemical phenotype similar to that of marginal zone cells: the neoplastic cells typically express CD20, Bcl2, and CD79a, but lack Bcl6. The plasma cells demonstrate monotypic light chain expression in 70% of cases (20). Cells express IgG more commonly than IgM, IgA, or IgD; this is in contrast to MZLs at other extranodal sites, which typically express IgM (17).

Other features distinguishing MALT lymphoma from extra-cutaneous disease include the absence of CXCR3 expression and presence of a Th2 type cytokine milieu (with higher levels of IL-4) in MALT lymphoma compared with extra-cutaneous MZL (30). In addition, the t(14;18)(q32;q21) IgH gene translocation is observed in fewer than 20% of cases (18). Abnormalities in the MALT1 gene, and trisomy 18 have been described in 25% of cases (18). The neoplastic cells have a post-germinal center phenotype and many cases demonstrate aberrant somatic hypermutation of PAX5, RhoH/TTF, cMYC and PIM1 (17,31). None of these genetic abnormalities occur with sufficient frequency to make their detection a clinically useful diagnostic parameter.

The differential diagnosis for MALT lymphoma includes pseudolymphomatous folliculitis, other forms of cutaneous lymphoid hyperplasia, primary cutaneous follicle center lymphoma (pcFCL),

primary cutaneous diffuse large B-cell lymphoma, leg type (pcDLBCL), and secondary cutaneous marginal zone lymphoma (sMZL).

3.4 CHARACTERISTICS COMPLICATING DISTINCTION OF PLF AND MALT LYMPHOMA

The primary challenge in diagnosing PLF is that this benign lymphoid proliferation may closely mimic lymphoid malignancies, both clinically and histopathologically (see **Table 1**). The most important entity in the differential diagnosis for PLF is primary cutaneous marginal zone lymphoma of mucosa-associated lymphoid tissue (MALT lymphoma or MALT lymphoma) (3,14,32). Because both PLF and MALT lymphoma present as an erythematous dome shaped nodule, they can be clinically indistinguishable. Although diagnosis thus depends largely on histopathologic analysis, biopsies of MALT lymphomas and PLF can also be remarkably difficult to differentiate: both may rarely present with clonal T-cell receptor rearrangements, atypical lymphocytes, and numerous admixed B and T cells (3,6,11,33). However, there are some distinguishing characteristics. First, MALT lymphomas are histopathologically characterized by dermal nodules of lymphoid cells with a proliferation of CD20+ marginal zone cells, sheets of plasma cells with monotypic immunoglobulin light chain expression (in 70% of cases); clonal immunoglobulin heavy chain (IgH) rearrangements are often detected (3,17,21) . Note that although MALT lymphomas are typically clonal for IgH, absence of detection of clonality does exclude a diagnosis of lymphoma; conversely, the presence of a clonal TCR does not rule out pseudolymphomatous folliculitis. Dutcher bodies (intranuclear pseudoinclusions), a preponderance of marginal zone cells, and sheets of plasma cells are strongly supportive of a diagnosis of MALT lymphoma, as is separation from the overlying epidermis by a grenz zone of uninvolved papillary dermis. PLF, on the other hand, presents as a dense patchy or diffuse

dermal infiltrate of small round T and B lymphocytes with hyperplastic, branching hair follicles invested with dendritic cells.

3.5 IMPORTANCE OF DISTINGUISHING PLF AND MALT LYMPHOMA

Although primary cutaneous marginal zone lymphomas are generally indolent and carry minimal risk of extra-cutaneous spread, it is still a lymphoma and necessitates substantial diagnostic testing and careful follow up (34). The National Comprehensive Cancer Network (NCCN) guidelines for diagnosis and workup of cutaneous B-cell lymphomas includes a history and physical, bloodwork, a CT of the chest, abdomen, and pelvis, and potentially bone marrow biopsy, PET-CT, peripheral blood flow cytometry, or serum protein electrophoresis (34). All of these tests are time consuming and costly. Incorrectly diagnosing a patient with PLF may result in under-staging and under-treatment of an actual lymphoma; likewise, an inaccurate diagnosis of MALT lymphoma would cause unnecessary testing, expense, stress, and treatment.

Given the clinical importance of distinguishing PLF and MALT lymphoma, improved histopathologic and immunohistochemical methods for differentiating these entities are necessary. The goal of these experiments is to identify improved Immunohistochemical markers for differentiating these two conditions.

Also of note, CD1a has been implicated in presentation of *Mycobacterium tuberculosis* antigens, and thus may play an important role in immunity against tuberculosis (TB) (35).

3.6 PROGRAMMED DEATH-1 (PD-1)

Programmed death-1 (PD-1) is a member of the immunoglobulin (Ig) superfamily of signaling proteins, along with CD28, T-lymphocyte associated antigen-4 (CTLA-4), inducible

costimulatory (ICOS), and B and T-lymphocyte attenuator (36–38). It was initially cloned as a cell-surface marker overexpressed in apoptosis (39). PD-1 has two known ligands, programmed death ligand 1 (PD-L1) and 2 (PD-L2), is expressed on T and B cells (37), and is generally considered a marker of follicular helper T-cells.

Some studies have found increased PD-1 signaling to be responsible for inhibition of T-cell receptor mediated T-cell proliferation and cytokine production (interferon gamma, IL-10, IL-4, and IL-2), suggesting that PD-1 is responsible for down-regulation of the immune response (40,41). Accordingly, PD-1 is thought to be implicated in mechanisms of peripheral tolerance (42). However, other studies have found that PD-1 is a co-stimulator of T-cell activation and proliferation (36–38), and that over-expression is associated with increased interferon gamma production (43). The reason for this discrepancy is currently unknown. It is now also believed to be associated with lymphocyte activation in T and B cells (39,44,45).

In human studies, normal PD-1 expression has been reported on follicular helper T-cells in the light zone of germinal centers in normal tonsils (46) and reactive lymph nodes (47). Increased levels of expression have been seen in various animal models of immunologic diseases including chronic infections (48,49) including HIV, *Helicobacter pylori*, and schistosomiasis (50), and several immunologic malignancies. Deficiency of PD-1 in mouse models has been associated with lupus-like disease, auto-antibody mediated dilated cardiomyopathy, and lethal GVHD (51,52). These reports underscore the importance of PD-1 as a negative regulator of immune responses.

Regarding expression of PD-1 in human neoplasms, PD-1 expression has been documented in a variety of lymphoid malignancies, and it is thought that expression of its ligand, PD-1L, may promote cancer progression via inhibition of the host anti-tumor response (50). PD-1 expression on reactive infiltrating T cells has been reported in extranodal non-Hodgkins lymphoma (47,50) and neoplastic B-cells in CLL/SLL (47). Expression in nodal NHLs appears to be minimal (47).

Increased levels of expression have also been reported in adult T-cell leukemia (53), angioimmunoblastic T-cell lymphoma (54,55), and Sezary syndrome (SS) (43,56), as well as some cases of lymphomatoid papulosis and mycosis fungoides (57). In studies regarding Sezary syndrome, it appears that increased levels of PD-1 may attenuate the immune response to neoplastic cells (43).

Blockade of PD-1 may help increase immune activity, enhance cytotoxic T-cell function, mediate cytokine production, improve T-cell recruitment, and ultimately reduced spread of tumor cells. The development of new PD-1 inhibitors thus makes this protein of particular interest in the study and treatment of human malignancies, both hematologic and non-hematologic (58).

Increased levels of PD-1 have been noted in a rare, indolent T-cell lymphoma known as primary cutaneous small/medium T-cell lymphoma (pcSMTCL) (57,59,60). It has been suggested that PLF may be related to or a variant of pcSMTCL. PD-1 expression has previously been examined in MALT lymphomas (56), but to the best of our knowledge had not been examined in PLF or other forms of CLH. It is on this basis that PD-1 was selected for inclusion in this study.

3.7 CLUSTER OF DIFFERENTIATION 1A (CD1A)

CD1a, CD1b, CD1c, CD1d, and CD1e are a members of the family of CD1 proteins, a class of proteins with limited homology to the MHC I family. These proteins are expressed as polypeptide heterodimers, made up of a heavy chain of CD1 bound noncovalently to the β2 microglobulin light chain (61). The CD1a protein contains a groove that permits binding to the T cell receptor, and a pocket capable of binding to lipids (61). CD1a is responsible for the presentation of lipid, glycolipid, and lipopeptide antigens to T cells (35), and thus may allow CD1a+ to report to T cells any alterations in lipid content that may occur in the setting of inflammation, infection, and malignancy (62). This protein is almost solely restricted to professional antigen presenting cells (APCs), including myeloid dendritic cells and Langerhans cells (35).

When CD1a expression in the skin is examined, a unique pattern emerges. The skin contains few CD1a+ APCs, primarily located in the epidermis (62,63). These cells are responsible for presentation of lipid autoantigens (62,63). CD1a auto-reactive T cells, located almost exclusively in the dermis (63), may play a significant role in skin homeostasis and remodeling of the basement membrane via induction of IL-22, IL-2, IL-13, and interferon-gamma (62).

Examination of CD1a expression in cutaneous lymphomas also yields interesting patterns. Mycosis fungoides, a cutaneous T-cell lymphoma composed of CD3+ CD4+ T cells, demonstrates a positive correlation between the number of T cells in the dermis and epidermis and the number of CD1a+ cells in the epidermis (63). Similar patterns have been observed in T-cell mediated inflammatory dermatoses and reactive conditions (64). In contrast, in cutaneous B-cell lymphomas demonstrated only a moderate CD1a+ infiltrate, which was generally confined to areas rich in T cells (63,65). Pigozzi *et al*. interpreted this pattern to mean that the neoplastic B-cell proliferation may damper the recruitment of CD1a cells into T-cell-rich areas (63). They propose that the neoplastic B cells may produce cytokines that inhibit CD1a+ DC recruitment, thus diminishing the local T-cell mediated anti-tumor response (63).

CD1a was selected for inclusion in this study given that increased numbers of CD1a+ dendritic cells within the hair follicle epithelium is an important diagnostic characteristic of PLF. This study makes a novel addition to the literature because CD1a expression patterns had not been previously examined in MALT lymphomas.

3.8 S-100

The S-100 proteins are a family of small, 10-12 kDa Ca2+ binding proteins with EF-hand domains, named for their solubility in 100% ammonium sulfate solution (66). The majority of the genes encoding this group of proteins are clustered on human chromosome 1q21 (67), a region known as the epidermal differentiation complex (68). There are at least 20 known members of the S-100 families in humans (68).

Given the function of calcium ions in the control and regulation of numerous biological processes, S-100 proteins are thought to be responsible for aspects of signal transduction and regulation of proliferation, apoptosis, transcription, and differentiation (67,69). These proteins are known to form homodimers, heterodimers, and oligodimers, enhancing their functional repertoire (70).

This marker was selected for inclusion in our study because the presence of S-100+ dendritic cells within the hair follicle epithelium had previously been reported. We sought to determine if the presence of S-100+ dendritic cells in the hair follicle epithelium could differential PLF from MALT lymphoma.

Of note, the antibody used in these studies can detect S-100-expressing langerhans cells (LC), dermal dendritic cells (DC), and melanocytes. Differential identification depends on the antigen retrieval technique used. Our current technique identifies S100+ DCs.

3.9 STATE OF THE FIELD

The ability to accurately and rapidly differentiate between the non-neoplastic proliferation PLF and MALT lymphomas is of tremendous importance to the diagnosis and treatment of patients and the maintenance of their wellbeing. At this time, differentiation largely depends on histopathologic pattern recognition, with few immunohistochemical markers. While prior reports suggest an increase in S-100+ and CD1a+ dendritic cells in the hair follicle epithelium of PLF, similar studies have not been performed in MALT lymphoma and other forms of CLH. We seek to identify widely available immunohistochemical markers useful in parsing this differential diagnosis.

3.10 PURPOSE OF INQUIRY

It has been suggested that PLF is a form of primary cutaneous small/medium T-cell lymphoma (pcSMTCL) or cutaneous lymphoid hyperplasia (CLH). The presence of a dense dermal lymphocytic infiltrate also raises concern for MALT lymphoma. Hence, we sought to identify immunohistochemical markers that might help parse this differential diagnosis.

PD-1 was included given reports of increased numbers of PD-1+ follicular helper T-cells in pcSMTCL. Further, PD-1 has not been previously examined in PLF. CD1a and S100 were included in this study given that the presence of S-100+ and CD1a+ dendritic cells within the hair follicle epithelium are considered supportive of a diagnosis of PLF but have not been studied in MALT lymphoma.

Based on these findings, we hypothesize that expression patterns of PD-1, CD1a, and S-100 in paraffin-fixed tissue samples may be helpful in distinguishing PLF, MALT lymphoma, and CLH. To examine the diagnostic value of immunohistochemical staining for these markers we examined staining patterns in 31 unique tissue samples including 13 skin biospies from 11 patients with MALT lymphoma, 12 skin biopsies from 9 patients with PLF, and 6 skin biopsies samples from 5 patients with CLH.

4 MATERIALS AND METHODS[3]

4.1 PATIENTS

Paraffin-embedded skin biopsies and/or glass slides from 9 patients with PLF, 11 patients with MALT lymphoma, and 5 patients with CLH were identified from the archives of the Dermatopathology Unit of the Massachusetts General Hospital Pathology Service and the private consultation files of the authors (Nancy L. Harris, Judith A. Ferry, Lyn M. Duncan). Multiple skin biopsy samples were available for each of three of the patients with PLF, one of the patients with MALT lymphoma, and one of the patients with CLH, yielding a total of 11 tissue samples of PLF, 13 of MALT lymphoma, and 6 of CLH. All cases had been previously reviewed by expert dermatopathologists and hematopathologists. MALT lymphomas were diagnosed based on the criteria laid out by the World Health Organization-European Organization of Research and Treatment of Cancer (WHO-EORTC) (17,71). Cases of PLF were identified histopathologically based on the presence of hyperplastic, irregular hair follicles containing S-100+ and CD1a+ dendritic cells and a nodular to diffuse dermal mixed lymphocytic infiltrate lacking significant atypia. In all instances, cases were only excluded if tissue for further immunohistochemical studies or necessary slides were unavailable. Clinical lesion location (eg. head/neck versus trunk) was not a selection criterion.

All patients diagnosed with MALT lymphoma and for whom follow-up data were available were staged as was considered clinically appropriate, including via physical examinations, blood counts, and computed tomography. No patients showed any evidence of extra-cutaneous disease at the time of diagnosis. Clinical characteristics examined included sex, age, clinical diagnosis, solitary or multiple lesions, anatomic location, tumor size, and duration of the lesion.

[3] The text of this section is derived from the paper: Goyal *et al*. "PD-1, S-100, and CD1a Expression in Pseudolymphomatous Folliculitis, Primary Cutaneous Marginal Zone B-cell Lymphoma (MALT lymphoma), and Cutaneous Lymphoid Hyperplasia." *Journal of Cutaneous Pathology*, Dec 11, 2014.

4.2 HISTOLOGY AND IMMUNOHISTOCHEMISTRY

Sections from all biopsies were routinely stained with hematoxylin/eosin (H&E). Histopathologic features examined included lesion size, architectural pattern, lymphocytic infiltration of pilosebaceous units, hair follicle morphology and irregularity (activation), grenz zone, presence and architecture of lymphoid follicles (defined as aggregates of B cells organized with a follicular dendritic cell meshwork, often including a germinal center), zones of plasma cells, and marginal zone cells.

Initially performed for diagnostic work ups, CD3 and CD20 immunostains were available for all tissue samples. Other immunohistochemical stains varied from sample to sample and included reagents to detect CD2, CD4, CD5, CD7, CD8, CD10, CD21, CD30, CD68, CD79a, Bcl2, Bcl6, Ki-67, kappa light chain, and lambda light chain. Immunohistochemical staining was performed as previously described.(72) In most cases, T-cell receptor and immunoglobulin heavy chain rearrangement were assessed by polymerase chain reaction after DNA extraction from the paraffin tissue blocks per established techniques.(72) For the purpose of this investigation, immunohistochemical staining for PD-1 (Z0311, Dako, Carpinteria, CA), CD1a (PA0235, Cell Marque, Rocklin, CA), and S-100 (315M-96, Cell Marque, Rocklin, CA) immunostains were performed on formalin-fixed paraffin embedded tissue sections (73,74). Control lymphoid tissue was present on the majority of slides; slides for which control tissue stained aberrantly were excluded or remade. Not all cases were stained for all markers due to limitations in the specimen size and block availability.

PD-1+ cells in the context of CD3+ T cells were evaluated immunohistochemically in 7 biopsies of PLF, 7 of MALT lymphoma, and 6 of CLH. The percentage of cells expressing PD-1 was

evaluated as previously described by Cetinozman et. al.(56) We added the use of ImageJ technology to provide a more quantitative estimate as follows: four photos of representative areas of each slide were taken at 60x magnification. The positive and negative cells in each photo were manually counted using ImageJ (Bethesda, MD) and the percentage of PD-1 positive cells averaged across the four images. The number of CD3+ cells was assessed similarly and the ratio of PD-1 to CD3 positive cells was then calculated.

Immunohistochemical stains for S-100 and CD1a to identify dendritic cell distribution were performed on twelve cases of PLF, thirteen cases of MALT lymphoma and 6 cases of CLH. The pattern of staining of S-100 and CD1a was categorized as within the hair follicle epithelium (intra-follicular), interstitial throughout the dermis, or peripheral about the lymphoid aggregates or excluded from reactive lymphoid follicles and scored on a scale of 0-3, 0 representing no staining and 3 indicating many dendritic cells with intense staining in the specified location.

4.3 STATISTICAL ANALYSIS

Statistical analysis was performed using Student's *t*-test, Fisher's exact test, and 1-way ANOVA test. P values <0.05 were considered significant.

5 RESULTS[4]

5.1 PATIENT CHARACTERISTICS

The cases examined included twelve biopsies from 9 patients with PLF, 13 biopsies from 11 with MALT lymphoma and 6 from 5 patients with CLH. Detailed information on patient age and gender, as well as lesion location, size, recurrence, treatment, and follow-up are provided in **table 2**. There was no statistically significant difference in patient age or gender. While all cases of PLF occurred on the head and neck, MALT lymphoma and CLH were more likely to occur on the trunk or extremities ($p < 0.0001$ and $p < 0.01$, respectively) (**Table 3**).

5.2 HISTOPATHOLOGY AND IMMUNOHISTOCHEMISTRY

While PLF, MALT lymphoma and CLH were difficult to distinguish based on examination of the H&E stained sections alone, some characteristic features were observed. All cases of PLF demonstrated hair follicles with irregularly shaped hyperplastic epithelial structures in the dermis (**Figure 1**). Lymphoid follicles and zones of plasma cells were most commonly observed in MALT lymphoma, and light chain restriction (12/13 cases, 7 kappa, 5 lambda) was used as a diagnostic criterion in MALT lymphoma (and was not seen in PLF or CLH). Neither hair follicle activation nor light chain restriction were seen in CLH.

[4] Portions of the text of this section are excerpted from the paper: Goyal *et al*. "PD-1, S-100, and CD1a Expression in Pseudolymphomatous Folliculitis, Primary Cutaneous Marginal Zone B-cell Lymphoma (MALT lymphoma), and Cutaneous Lymphoid Hyperplasia." *Journal of Cutaneous Pathology*, Dec 11, 2014. Excerpted portions include the text of sections 3.1, 3.2, 3.3, 3.4, Table 3, and Table 5. Tables 1, 2, 4, and 6 and any text pertaining to them were not included in that manuscript.

5.3 PD-1 AND CD3 STAINING

The lymphoid infiltrate in all cases was composed of numerous admixed CD3+ T and CD20+ B cells. In PLF, CD3+ T cells comprised 57-78% of the infiltrate (mean 69%), whereas 38-70% (mean 54%) of the small round lymphocytes stained positively for PD-1 (**Figure 1**). In MALT lymphoma, CD3+ T cells composed 29-80% of the infiltrate (mean 67%), with 6-21% (mean 16%) of the small round lymphocytes staining positively for PD-1. In CLH, CD3+ T cells accounted for 53-64% of the infiltrate (mean 61%), and 13-30% (mean 24%) of the small round lymphocytes stained positively for PD-1 (**Table 4**).

The proportion of reactive CD3+ T cells expressing PD-1, calculated as the PD-1/CD3 ratio (56), ranged from 65-97% (mean 78%) in PLF, to 17-34% (mean 23%) in MALT lymphoma, and 21-56% (mean 40%) in CLH (**Table 5, Figure 2**).

5.4 S-100 AND CD1A STAINING

S-100+ dendritic cells were present in the hair follicle epithelium in all cases with hair follicles present, including 12 cases of PLF, 3 cases of MALT lymphoma and 2 cases of CLH. The distribution of S-100+ dendritic cells in the dermis was examined. An interstitial pattern throughout the dermal infiltrate was observed in all 11 cases of PLF, in 6 of 13 cases of MALT lymphoma and 5 of 6 cases of CLH. Localization of S-100+ dendritic cells at the periphery of the lymphoid nodules was not seen in PLF (0/10 cases, 1 case was a small punch biopsy without the edge of a lymphoid nodule) or CLH (0/6) and was only seen in 1 case of MALT lymphoma (1/7 cases) (**Figure 3, Table 4**).

A prominent population of CD1a+ dendritic cells was observed in the hair follicle epithelium of all cases of PLF (10/10 cases), in the 2 cases of MALT lymphoma with hair follicle epithelium, and in both cases of CLH with hair follicles present (**Figure 3**). CD1a+ dendritic cells were

observed throughout the dermis in an interstitial pattern in most cases of PLF (9/10), occasionally in MALT lymphoma (6/13 cases) and most cases of CLH (5/6 cases). CD1a+ dendritic cells were not observed localized to the periphery of lymphoid nodules in cases of PLF with lymphoid nodules (0/7 cases), but were seen in the majority of cases of MALT lymphoma (11/13 cases), and occasionally seen in CLH (2/6 cases) (**Table 4**). In cases with reactive lymphoid follicles (most cases of MALT lymphoma and rare cases of CLH), the CD1a+ cells were absent in lymphoid follicles (**Figure 3f**).

6 DISCUSSION[5]

Although the term pseudolymphomatous folliculitis (PLF) was initially coined by McNutt in 1986 (15), it was not formally established as a distinct subtype of cutaneous lymphoid hyperplasia (CLH) until 1999 (4). In their 1999 paper, Arai *et al*. distinguished PLF on the basis of its characteristic lymphocytic invasion into the pilosebaceous unit epithelial lining and accompanying architectural distortion of the hair follicle epithelium (4). This was a notable observation because previously, adnexal invasion was considered a characteristic suggestive of lymphoma rather than a benign lymphoid hyperplasia. Since these seminal works were published, many case reports and small case series have further demonstrated the unique clinical and histopathologic traits characteristic of PLF (3–14).

6.1 PSEUDOLYMPHOMATOUS FOLLICULITIS (PLF) VS. MALT LYMPHOMA

While MALT lymphomas are generally benign and carry minimal risk of extracutaneous spread, a diagnosis of MALT lymphoma can be significantly detrimental to a patient's emotional and financial life. An inaccurate diagnosis of PLF can result in under-treatment of a lymphoma, while an inaccurate diagnosis of MALT lymphoma can resign a patient to thousands of dollars of testing and incalculable stress and anxiety. Accurate differentiation of cutaneous lymphoid hyperplasias (particularly pseudolymphomatous folliculitis) from MALT lymphoma can be integral to patient care clinically, emotionally, and financially.

However, resolving this differential histopathologically can be markedly challenging. Several characteristics may be common to both entities. First, while hair follicle epithelium hyperplasia is

[5] The text of this section has been derived from "PD-1, S-100, and CD1a Expression in Pseudolymphomatous Folliculitis, Primary Cutaneous Marginal Zone B-cell Lymphoma (MALT lymphoma), and Cutaneous Lymphoid Hyperplasia." *Journal of Cutaneous Pathology*, Dec 11, 2014.

considered characteristic of PLF, and found in all of our cases, such hyperplasia has also been observed in cutaneous B-cell lymphoma (6). Second, although lymphoid follicles and zones of plasma cells and neoplastic B cells are characteristic of MALT lymphoma (11), cytologic atypia may be very subtle. Third, to add further complication, although MALT lymphoma is a B-cell lymphoma, in some cases, the number of T cells may far outnumber B cells (27,75); conversely, cases of PLF may demonstrate a preponderance of B cells. Hence, the proportion of T cells vs. B cells is not a reliable means of differentiation (3,4,6). Fourth, while the detection of light chain restriction in plasma cells (via immunohistochemistry or in situ hybridization) is diagnostic \of MALT lymphomas and present in 70% of cases, this may be absent in 30% of cases (27); the remainder of these cases demonstrate clonal immunoglobulin heavy chain (IgH) rearrangements via PCR. IgH clonality is an important asset in parsing this differential given that it is very rarely observed in PLF and CLH. In these experiments, three biopsies of PLF underwent IgH gene rearrangement testing; all three were negative. Also of assistance, while T-cell receptor (TCR) rearrangements are rarely found in MALT lymphoma (3,6,33), TCR gene rearrangements may be found in PLF and CLH. In our study, five biopsies of PLF underwent TCR gene rearrangement studies; of these, two demonstrated clonal TCR rearrangement.

6.2 Pseudolymphomatous Folliculitis (PLF) vs. Cutaneous Lymphoid Hyperplasia (CLH)

Due to significant overlap in clinical and histopathologic features between CLH and PLF, some still consider PLF to be a clinically distinct form of CLH. Although PLF and CLH both clinically present as an erythematous non-ulcerated nodule composed of a dense dermal lymphoid infiltrate, occasionally with lymphoid follicles, there are some differentiating features. PLF typically occurs on the face of young males, and demonstrates distinctive hair follicle epithelium hyperplasia. In support of the distinction between PLF and CLH, we found that T cells in PLF more commonly express PD-1 (65-97%) than those in CLH (21-57%). While the specific

immunologic mechanisms for these differences are unclear, these data support the hypothesis that PLF and CLH are distinct entities.

6.3 PD-1 EXPRESSION

Given the clinical and histopathologic difficulties we are faced with in differentiating MALT lymphoma, PLF, and other forms of CLH, we performed these studies with the intent of identifying new immunohistochemical tools that would allow us to better assess this differential.

Here, we report that there is a significant difference in PD-1 expression by small round lymphocytes in PLF, MALT lymphoma, and CLH. The mean percentage of lymphocytes expressing PD-1 was 54%, 16%, and 24%, respectively. Thus, the proportion of lymphocytes expressing PD-1 in PLF was significantly higher than in MALT lymphoma ($p<0.0001$). Similarly, the proportion of lymphocytes expressing PD-1 in CLH was also higher than MALT lymphoma ($p=0.03$). However, there was no observed difference in CD3+ T cell density among the three groups ($p=0.49$).

When we assessed the proportion of CD3+ T cells expressing PD-1, there were several significant differences between the three groups. First, PLF demonstrated a higher ratio of CD3+ cells expressing PD-1 (mean 78%), than MALT lymphoma (mean 25%, $p<0.0001$) or CLH (mean 40%, $p<0.002$). Similarly, the PD-1/CD3 ratio in CLH was also higher than that in MALT lymphoma ($p<0.01$). These findings are in accordance with the recent study by Cetinozman *et al*. In that study, they report that MALT lymphomas show expression of PD-1 in 10% of all cells and 20% of T cells (56). An additional important observation, in our cases of MALT lymphoma, CD1a+ dendritic cells were generally concentrated adjacent to areas of high PD-1+ T cell density.

PD-1 is predominantly expressed on activated follicular helper T cells in the light zone of germinal centers in normal tonsils (46) and reactive lymph nodes (47). Follicular helper T cells

are critical to the maintenance and regulation of germinal center B cell differentiation into memory B cells and plasma cells (76). It is thought that high levels of PD-1 expression of follicular helper T cells may limit T-cell signaling directly downstream of the T cell receptor and diminish cytokine secretion (76).

PD-1 expression patterns in B-cell lymphomas have been the focus of a handful of recent papers, including Cetinozman et al. and Mitteldorf et al. These groups have shown that there is a greater density of PD-1+ T cells in MALT lymphoma than in primary cutaneous follicle center lymphoma (pcFCL) or cutaneous diffuse large B-cell lymphoma (56,77). Furthermore, other groups have discovered that increased density of PD-1+ T cells in systemic B-cell lymphomas correlates with improved survival rates (78).

PD-1 is of particular interest in the field of oncology because of the recent development of new PD-1 inhibitors. PD-1 blockade may help enhance cytotoxic T-cell function, mediate cytokine production, increase immune activity, improve T-cell recruitment, and reduce the spread of tumor cells. Expression of PD-1's ligand, PD-1L, may promote cancer progression via inhibition of the host anti-tumor response (50).

In summary, the expression pattern we observed is consistent with that reported in the literature, with PD-1 primarily expressed on CD3+ T-cells (47). This conclusion is limited by the fact that we did not perform co-staining, but rather correlated staining patterns. Low levels of expression on neoplastic B-cells cannot be ruled out. The suggestion that PD-1 is implicated in immune tolerance, and the observation that increased levels of expression are associated with other B-cell neoplasms (47), suggest that the infiltrating reactive PD1+ CD3+ T-cells may play a role in facilitating the expansion of B-cell populations.

6.4 CD1A EXPRESSION PATTERNS

One of the primary characteristics of PLF, as described by Arai et al. (3), is the proliferation of CD1a+ dendritic cells within the hair follicle epithelium. Given the pre-existing diagnostic significance of this marker (3,4), we elected to include this marker in our study. Expression of this transmembrane cell-surface protein has been reported on dendritic cells, immature T cells, activated monocytes, and a subset of B cells (65).

While we did not identify a difference in CD1a expression on dendritic cells located in the hair follicle epithelium between PLF, MALT lymphoma, and CLH, we did note a marked difference in the distribution of CD1a+ cells in the dermis. Both PLF and CLH showed the presence of CD1a+ dendritic cells throughout the dermal infiltrate. In MALT lymphomas, on the other hand, the CD1a+ dendritic cells were located around the periphery of the neoplastic B-cell aggregates, excluded from the areas of B cells and restricted to areas of high T-cell density. Previous studies of cutaneous B-cell lymphomas have demonstrated a moderate contingent of CD1a+ cells, limited to areas rich in T cells (63,65). This has been taken to mean that CD1a+ cell recruitment to T-cell rich areas may be hampered by the neoplastic B-cell proliferation, possibly via secretion of cytokines (63).

CD1a+ cells were absent from reactive lymphoid follicles and few were found scattered throughout the epidermis in MALT lymphoma. This is markedly similar to the distribution of CD123+ plasmacytoid dendritic cells in MALT lymphomas as noted by Kempf et al. (79). The presence of CD1a+ dendritic cells scattered throughout the dermis seen in PLF and CLH was consistent with staining patterns reported in other cutaneous pseudolymphomas (65).

These findings suggest that patterns of CD1a staining may be a valuable tool in differentiating MALT lymphomas from other cutaneous lymphoid proliferations: a peripheral staining pattern around a nodular lymphoid proliferation is consistent with a diagnosis of MALT lymphoma, while an interstitial staining pattern is more suggestive of PLF or CLH. The biological significance of these distribution patterns remains to be further investigated.

6.5 S-100 EXPRESSION PATTERNS

The presence of S-100+ dendritic cells within the hair follicle epithelium of PLF has been reported as an important diagnostic characteristic of PLF (3,4); hence, we elected to analyze this immunohistochemical marker in our study in order to assess if expression patterns varied between PLF, MALT lymphoma and CLH. Although we did identify numerous intrafollicular S-100+ dendritic cells in PLF, we were unable to identify any statistically significant difference in the number of intrafollicular S-100+ dendritic cells among the three entities. Similarly, we also did not identify any significant difference in the dermal distribution pattern of S-100+ cells.

It is notable that CD1a and S-100 staining patterns were significantly different, particularly in MALT lymphoma: CD1a+ dendritic cells were located around the periphery of lymphoid nodules but excluded from the nodules themselves, while S-100+ cells were scattered throughout the infiltrate. Given the contrast in staining patterns, it is possible that CD1a and S-100 are staining different populations of dermal dendritic cells.

Thus, although historically S-100 staining was considered an important characteristic in diagnosing PLF, we find that it is not helpful in discriminating PLF, MALT lymphoma, and CLH.

6.6 LIMITATIONS OF THIS STUDY

There are several important limitations to this study. First, our conclusions are based on the assumption that each sample was initially given a correct diagnosis. Diagnoses were based on expert histopathologic analysis, including analysis of hair follicle morphology, the quality of the dermal lymphocytic infiltrate, IgH clonality studies, and analysis of kappa/lambda light chain restriction. To help reduce the likelihood of incorrect diagnosis, all cases were reviewed by experts in cutaneous oncology, dermatopathology, and hematopathology prior to initiation of our analysis.

Given that this is a retrospective study it is subject to selection bias. To attempt to alleviate this concern, consecutive cases of PLF, MALT lymphoma, and CLH from the records of the Massachusetts General Hospital Dermatopathology unit were pulled. Cases were only excluded if insufficient tissue was available for study.

Finally, although these observations promise to be diagnostically helpful, our conclusions are minimally elucidative about the pathology of these lymphoid proliferations. Substantial investigation remains to be done.

7 CONCLUSIONS

Our immunohistochemical assessment found that there is a statistically significant increase in PD-1 expression by T lymphocytes in PLF as compared to MALT lymphoma. We also found that the staining pattern of CD1a was highly informative—MALT lymphoma was significantly more likely to demonstrate staining of CD1a+ cells around the periphery of aggregates of lymphocytes as compared to PLF or CLH. In contrast, PLF and CLH were more likely to demonstrate an interstitial distribution of CD1a+ cells throughout the dermal lymphoid infiltrate. There was no significant difference in S-100 staining among the three entities.

Although the findings in this study may be limited by selection bias and the fact that the “gold standard” diagnosis is based on expert histopathologic analysis, our data suggest that PD-1 and CD1a may be useful adjuncts in distinguishing these dense dermal lymphocytic proliferations.

8 FUTURE DIRECTIONS

This study unveiled an as-of-yet unknown difference between PLF, MALT lymphoma, and other forms of cutaneous lymphoid hyperplasia—the apparent exclusion of CD1a cells from neoplastic lymphoid aggregates of MALT lymphoma. Further investigations are necessary to understand this differential distribution.

The utility of this marker must further be evaluated in prospective studies. At the Massachusetts General Hospital Dermatopathology Unit, PD-1 and CD1a have been added to the diagnostic panel for cases of dense cutaneous lymphoid infiltrates concerning for MALT lymphoma.

Additional examination of other immune markers may be helpful, both in identifying additional diagnostic targets and in helping elucidate the biology underlying these conditions. Considerations include examination of T-cell subsets using CD4 and CD8; examination of other dendritic cell subsets, including CD123 for plasmacytoid dendritic cells; and PD-1 ligand 1 (PD-L1) and PD-1 ligand 2 (PD-L2) to help assess the significance of the increased numbers of PD-1+ T-cells in PLF.

9 SUMMARY

In this study we examined the immunophenotype and composition of the cellular infiltrate in skin biopsy samples from 9 patients with PLF, 11 patients with MALT lymphoma, and 5 patients with cutaneous lymphoid hyperplasia. The clinical, histopathologic, and immunohistochemical characteristics of each of these cases were reviewed with the goal of determining the usefulness of PD-1, S100, and CD1a in differentiating these three entities. We performed additional analysis to examine the utility of three Immunohistochemical markers: 1) the proportion of T cells expressing PD-1, 2) the pattern of expression of CD1a, and 3) the pattern of expression of S100.

We found that the density of PD-1+ T cells and the distribution of CD1a+ dendritic cells were helpful features in distinguishing these three diagnostic entities that may mimic one another histopathologically. Increased PD-1 expression by T-cells was more often observed in PLF or CLH as compared to MALT lymphoma. Expression of CD1a at the periphery of the dermal lymphoid infiltrate was seen primarily in MALT lymphoma, while an interstitial pattern is more consistent with PLF or CLH. Finally, we found that S100 staining was not helpful in parsing this differential diagnosis.

10 TABLES

10.1 TABLE 1

Clinical and histopathologic characteristics of pseudlymphomatous folliculitis (PLF), MALT lymphoma, and cutaneous lymphoid hyperplasia (CLH). Adapted from Goyal et al. (80).

	PLF	**MALT lymphoma**	**CLH**
Clinical presentation	Solitary, violaceous 5-30 mm domed nodule without ulceration or scaling	Solitary or multiple erythematous-to-violaceous plaques, nodules, or tumors	Firm skin-colored to erythematous to violaceous nodule/tumor or infiltrated plaques
Location	Scalp, face, or upper trunk	Trunk, extremities	Face (70%), chest (36%), upper extremities (25%)
Associated symptoms	None	None	Often at sites of bites, trauma, vaccinations, tattoos
Median age	50s	60s	30s
Gender predilection	Male	Male	Female
Common Treatment	Excision, spontaneous resolution	Radiotherapy, excision, intralesional steroids	Excision, intralesional steroids
Likelihood of extracutaneous spread	None	5%	None
5-year survival	100%	100%	100%
Cellular morphology	Polymorphous lymphocytes	Centrocyte-like marginal zone B cells, variable	Polymorphous lymphocytes with admixed histiocytes,

		lymphoplasmacytoid and plasma cells, rare immunoblasts	eosinophils, and plasma cells
Distribution of lymphoid infiltrate	Dense, patchy or diffuse, dermal infiltrate of small round lymphocytes	Dense, nodular, dermal and subcutaneous; intact and colonized reactive lymphoid follicles may be present	Nodular or diffuse infiltrate with reactive follicles
Distinctive features	Hyperplastic, branching and irregularly shaped hair follicles with intra-follicular dendritic cells	Sheets of marginal zone B cells and aggregates of lymphoplasmacytoid cells;occasional mature plasma cells;rare immunoblasts; occasionally frequent non-neoplastic T cells	No abnormal follicle architecture, no light chain restriction
Immunohistochemical markers	CD1a+ and S-100+ dendritic cells in the hair follicle epithelium	CD20+ PAX5+ CD79a+ Bcl6- CD10- Bcl2+ MUM1+ CyclinD1- CD5- IgM -/+	Admixed infiltrate of CD3+ T cells that often predominates. CD20+ B cells, occasionally with well-formed reactive lymphoid follicles
Molecular genetic features	No clonally rearranged *IGH* May see rearranged TCR in reactive/oligoclonal	*IGH, MALT1, API2*, and *FOXP1* translocations (81)	No clonally rearranged *IGH* May see rearranged TCR in reactive/oligoclonal

	responses in the skin		responses in the skin
Immunoglobulin (Ig) clonality	Polyclonal Ig	Monoclonal cytoplasmic Ig	Polyclonal Ig
Light chain restriction	Absent	Often present (can be demonstrated in up to 70% cases)	Absent

10.2 TABLE 2

Data on patient age, gender, lesion location, multifocality, size, recurrence, treatments, and duration of follow-up.

	Gender	Age	Location	Multifocal	Size	Recurrence	Treatment*	Duration of follow-up
Pseudolymphomatous folliculitis (PLF)								
1	M	53	L. nose	No	0.3 cm	No	PO steroids	9 years
2	F	46	R. nose	No	0.2 cm	N/A	N/A	N/A
3	M	17	L. nose R. cheek	Yes	0.5 cm, 1.5 cm	Single	1) EX 2) ILS	6 months
4	M	58	Nasal bridge x 2	No	1-2 cm	Single	1) BX 2) ILS	6 years
5	M	65	Scalp	No	N/A	N/A	EX	N/A
6	M	47	L. temple	No	N/A	N/A	EX	N/A
7	M	69	R. cheek x 2	No	1.5 cm	No	ILS, BX, EX	6 months
8	M	57	R. parietal scalp	No	2 cm	No	Bx	2 months
9	M	60	Forehead	No	0.8 cm	Single	ILS	7 months
MALT lymphoma								
1	F	38	L. arm	Yes	0.5 cm	Multi	1) XRT 2+): ILS	4 years
2	M	80	R. arm	No	0.4 cm	No	XRT	5 years
3	M	55	R. thigh x 2	No	1 cm	Multi	1) ILS. 2+): PO steroids, XRT	7 years
4	F	81	R. upper arm x 2	Yes	1.5 cm	No clearance	Rituximab for concurrent sDLBCL	3 years

5	M	47	R. upper arm	No	0.4 cm	No	ILS and Bx	2 years
6	M	45	L. arm	Yes	0.5-1 cm	Multi	1) Topicals, ILS 2+): ILS, XRT	15 years
7	F	65	R. leg L. calf	Yes	2-4 cm	Multi	1) ILS, XRT 2+) ILS, XRT	5 years
8	F	74	R. arm	No	2 cm	N/A	N/A	N/A
9	F	74	R. shoulder	Yes	N/A	N/A	N/A	N/A
10	M	84	L. chest	No	N/A	N/A	N/A	N/A
11	F	75	L. forehead	No	1.3 cm	No	EX, XRT	3 months
Cutaneous lymphoid hyperplasia (CLH)								
1	F	68	L. chest	No	1.6 cm	N/A	N/A	N/A
2	F	36	L. forehead	No	0.5 cm	Single	1) ILS 2) ILS	9 years
3	F	60	R. trunk L. breast	Yes	N/A	N/A	N/A	N/A
4	M	77	L. shoulder	No	1.5 cm	No	ILS	5 years
5	M	49	L. back	Yes	0.5-1.5 cm	Multi	1) Topical, ILS, XRT 2+) Topical, ILS	7 years

*Complete clearance occurred unless otherwise noted.

sDLBCL—systemic diffuse large B-cell lymphoma, N/A—not available, ILS—Intralesional steroid injection, Bx—spontaneous clearance after biopsy, XRT—Radiation therapy, EX—excision, N—no, S—single, Multi—multiple, 1)—treatment of initial lesion, 2)—treatment of first recurrence, 2+)—treatment of multiple recurrences

10.3 TABLE 3

Summary of characteristics of patients with pseudolymphomatous folliculitis (PLF), MALT lymphoma, and cutaneous lymphoid hyperplasia (CLH).

	PLF	MALT	CLH
Number of patients	9	11	5
Age	17-69 years Mean: 52 years	38-84 years Mean: 65 years	36-77 years Mean: 48 years
M/F	8/1	5/6	2/3
Size	0.2-2 cm Mean: 1.03 cm	0.4-4 cm Mean: 1.16 cm	0.5-1.6 cm Mean: 1.15 cm
Duration of follow up	2 months-9 years Mean: 2.8 years	3 months-15 years Mean: 5.5 years	5-9 years Mean: 7 years
Number of lesions biopsied	12	13	6
Lesion Locations			
Head	12	1	1
Trunk/Extremities	0	12	5

10.4 TABLE 4

Percentages of cells staining positively for CD3 and PD-1, and ratio of PD-1/CD3 positive cells.

The number of cells staining positively and negatively for PD-1 and CD3 in each field analyzed. Four fields at 60x magnification (lettered a-d) were analyzed for each tissue sample. Percentage of cells staining positively for each marker was calculated for each field, as well as the ratio of PD-1+ to CD3+ cells in each field. The average for the four fields of each sample was taken to determine the percentage of cells staining positively for each marker for each individual tissue sample.

Patient #	Field #	PD 1+	PD 1-	Total	% PD1+	Avg.	CD3+	CD3-	Total	%CD 3+	Avg.	%PD1/ CD3
Pseudolymphomatous folliculitis												
1	**a**	224	136	360	62%	**69%**	215	91	306	70%	**77%**	**89%**
	b	227	93	320	71%		300	82	382	79%		
	c	246	111	357	69%		265	81	346	77%		
	d	247	96	343	72%		276	63	339	81%		
3a	**a**	161	197	358	45%	**38%**	225	146	371	61%	**58%**	**65%**
	b	128	251	379	34%		219	172	391	56%		
	c	198	255	453	44%		225	141	366	61%		
	d	111	262	373	30%		199	165	364	55%		
3b	**a**	184	201	385	48%	**39%**	214	200	414	52%	**57%**	**68%**
	b	129	275	404	32%		253	148	401	63%		
	c	127	280	407	31%		225	129	354	64%		
	d	133	168	301	44%		127	128	255	50%		
4a	**a**	150	144	294	51%	**43%**	227	121	348	65%	**65%**	**67%**
	b	162	183	345	47%		215	117	332	65%		
	c	120	194	314	38%		225	131	356	63%		
	d	136	224	360	38%		200	104	304	66%		
4b	**a**	144	170	314	46%	**55%**	249	79	328	76%	**73%**	**76%**
	b	183	159	342	54%		263	84	347	76%		
	c	194	142	336	58%		253	128	381	66%		
	d	224	126	350	64%		256	89	345	74%		

6	**a**	332	123	455	73%	**71%**	379	117	496	76%	**73%**	**97%**
	b	335	111	446	75%		321	146	467	69%		
	c	275	165	440	63%		315	118	433	73%		
	d	308	115	423	73%		326	119	445	73%		
8	**a**	279	127	406	69%	**64%**	436	101	537	81%	**78%**	**81%**
	b	194	82	276	70%		294	74	368	80%		
	c	205	209	414	50%		301	102	403	75%		
	d	226	113	339	67%		325	93	418	78%		
Overall Average					**54%**					**69%**	**78%**	
MALT Lymphoma												
1	**a**	63	441	504	13%	**16%**	302	97	399	76%	**68%**	**24%**
	b	105	255	360	29%		268	68	336	80%		
	c	84	472	556	15%		203	163	366	55%		
	d	45	519	564	8%		198	123	321	62%		
2	**a**	89	382	471	19%	**17%**	349	86	435	80%	**77%**	**23%**
	b	67	296	363	18%		267	117	384	70%		
	c	66	362	428	15%		432	126	558	77%		
	d	75	385	460	16%		301	80	381	79%		
3	**a**	73	288	361	20%	**19%**	327	79	406	81%	**80%**	**23%**
	b	54	241	295	18%		251	73	324	77%		
	c	47	220	267	18%		212	67	279	76%		
	d	96	419	515	19%		313	48	361	87%		
3	**a**	126	361	487	26%	**22%**	227	126	353	64%	**64%**	**35%**
	b	127	304	431	29%		277	130	407	68%		
	c	44	300	344	13%		239	281	520	46%		
	d	104	378	482	22%		295	78	373	79%		
4	**a**	31	366	397	8%	**14%**	296	85	381	78%	**78%**	**17%**
	b	79	282	361	22%		347	96	443	78%		
	c	53	340	393	13%		288	83	371	78%		
	d	44	362	406	11%		314	92	406	77%		
5	**a**	17	584	601	3%	**6%**	84	329	413	20%	**30%**	**19%**

	b	16	442	458	3%		101	261	362	28%		
	c	36	347	383	9%		182	257	439	41%		
	d	32	449	481	7%		128	315	443	29%		
	Overall Average					**16%**					**66%**	**23%**
Cutaneous Lymphoid Hyperplasia												
1	**a**	54	261	315	17%	**13%**	179	102	281	64%	**63%**	**21%**
	b	41	427	468	9%		325	208	533	61%		
	c	68	353	421	16%		311	237	548	57%		
	d	34	311	345	10%		224	94	318	70%		
2	**a**	109	421	530	21%	**24%**	304	122	426	71%	**61%**	**39%**
	b	145	409	554	26%		226	185	411	55%		
	c	147	372	519	28%		303	100	403	75%		
	d	120	451	571	21%		195	250	445	44%		
3a	**a**	82	358	440	19%	**25%**	225	117	342	66%	**63%**	**40%**
	b	83	246	329	25%		175	148	323	54%		
	c	100	360	460	22%		270	133	403	67%		
	d	164	300	464	35%		273	138	411	66%		
3b	**a**	95	237	332	29%	**25%**	142	99	241	59%	**61%**	**40%**
	b	102	348	450	23%		196	111	307	64%		
	c	69	239	308	22%		171	84	255	67%		
	d	90	269	359	25%		128	100	228	56%		
4	**a**	130	280	410	32%	**30%**	142	187	329	43%	**53%**	**57%**
	b	138	380	518	27%		161	233	394	41%		
	c	105	235	340	31%		261	108	369	71%		
	d	109	259	368	30%		184	149	333	55%		
5	**a**	68	186	254	27%	**28%**	306	130	436	70%	**62%**	**45%**
	b	98	156	254	39%		207	127	334	62%		
	c	106	648	754	14%		172	160	332	52%		
	d	136	284	420	32%		226	126	352	64%		
	Overall					**24%**					**61%**	**40%**

	Average										

10.5 TABLE 5

Percentage of cells staining positively for PD-1, CD3, and the ratio of PD-1/CD3 positive cells in pseudolymphomatous folliculitis (PLF), MALT lymphoma, and cutaneous lymphoid hyperplasia (CLH). There is a statistically significant difference in the percentage of cells staining positively for PD-1 and the ratio of PD-1/CD3 positive cells among the three entities.

	N	%PD1+ (range)	%CD3+ (range)	PD1/CD3 (range)
PLF	7	54 (38-70)	69 (57-78)	78 (65-97)
MALT	6	16 (6-21)	67 (29-80)	23 (17-34)
CLH	6	24 (13-30)	61 (53-64)	40 (21-56)
		p <0.0001	p=0.49	p < 0.0001

10.6 TABLE 6

Quantification of staining patterns for CD1a and S-100 in pseudolymphomatous folliculitis (PLF), MALT lymphoma, and cutaneous lymphoid hyperplasia (CLH). Staining was scored on a 0-3 point scale, 0 being no staining and 3 being extremely prominent staining. Samples were scored independently by authors AG and LMD.

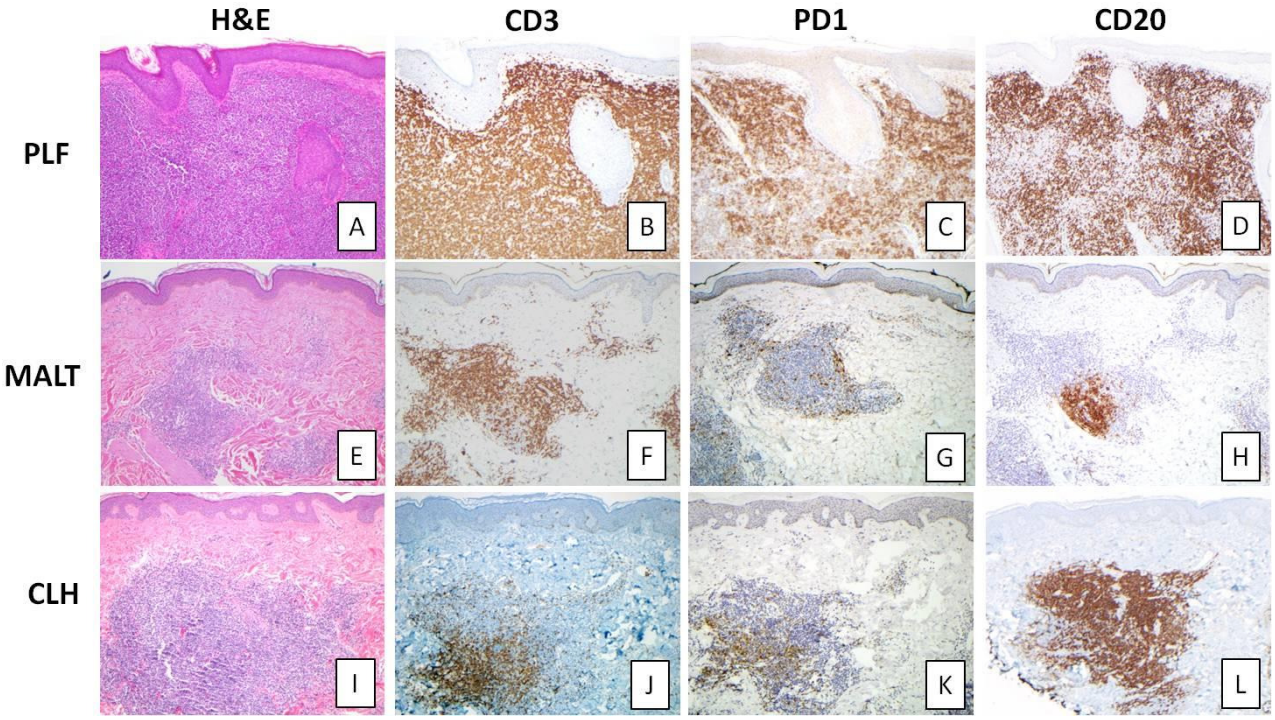
	CD1a			S-100		
Patient #	**Follicular**	**Interstitial**	**Peripheral**	**Follicular**	**Interstitial**	**Peripheral**
Pseudolymphomatous Folliculitis						
1	3	2	N/A	3	2	0
2	3	2	0	2	2	0
3a	2	1	N/A	2	2	N/A
3b	3	1	0	2	1	0
4a	2	0	0	1	1	0
4b	2	2	N/A	1	1	0
5	3	2	0	2	2	0
6	3	1	0	3	2	0
7a	N/A	N/A	N/A	1	2	0
7b	3	1	0	3	2	0
8	3	2	0	3	1	0
9	3	2	0	N/A	N/A	N/A
MALT lymphoma						
1	N/A	0	3	N/A	1	0
2	N/A	3	3	N/A	2	0
3a	N/A	0	3	N/A	1	0
3b	N/A	2	3	N/A	1	2
4a	N/A	0	0	N/A	0	0

4b	N/A	0	0	N/A	N/A	N/A
5	3	0	3	2	1	0
6	2	1	2	N/A	N/A	N/A
7	N/A	1	3	0	2	0
8	N/A	0	2	N/A	N/A	N/A
9	N/A	3	2	N/A	N/A	N/A
10	N/A	0	2	N/A	N/A	N/A
11	N/A	1	3	N/A	N/A	N/A
Cutaneous lymphoid hyperplasia						
1	3	2	0	2	1	0
2	2	0	0	0	0	0
3a	N/A	3	1	N/A	2	0
3b	N/A	3	0	N/A	1	0
4	N/A	2	2	N/A	0	0
5	N/A	3	0	N/A	2	0

11 FIGURES

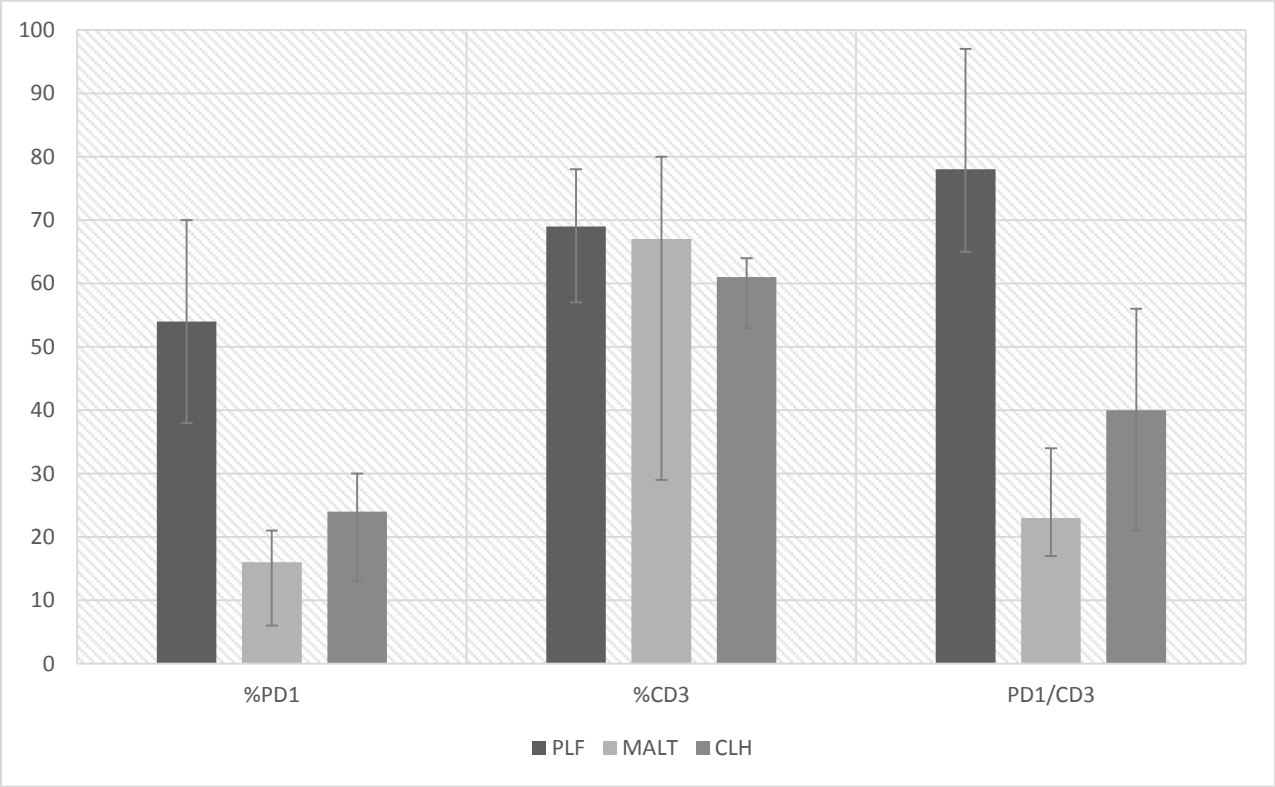
11.1 FIGURE 1

CD3 and PD-1 in pseudolymphomatous folliculitis (PLF), extranodal marginal zone lymphoma of mucosa-associated lymphoid tissue (MALT lymphoma), and cutaneous lymphoid hyperplasia (CLH). PLF v. CLH, with a dense superficial and deep dermal lymphocytic infiltrate (A, H&E, 10x), numerous CD3+ T cells (B, CD3, 10x) and many PD-1+ cells (C, PD-1, 10x). Numerous CD20+ B cells are present throughout the infiltrate (D, CD20, 10x). MALT lymphoma with dense dermal nodules of marginal zone B-cells and reactive T-cells (E, H&E, 10x), reactive CD3+ T cells within the nodular proliferation (F, CD3, 10x) and fewer than a third of the CD3+ T-cells express PD-1 (G, PD1, 10x). CD20+ cells are present as a dense aggregate in the nodular proliferation (H, CD20, 10x). CLH with a dense nodular mixed lymphohistiocytic infiltrate (I, H&E, 10x), a substantial population of CD3+ T-cells (J, CD3, 10x) and less than half of CD3+ T cells express PD-1 (K, PD-1, 10x). Many CD20+ B cells are present throughout the nodular lymphoid proliferation (L, CD20, 10x).


H&E
CD3
PD1
CD20
PLF
MALT
CLH
A
B
C
D
E
F
G
H
I
J
K
L

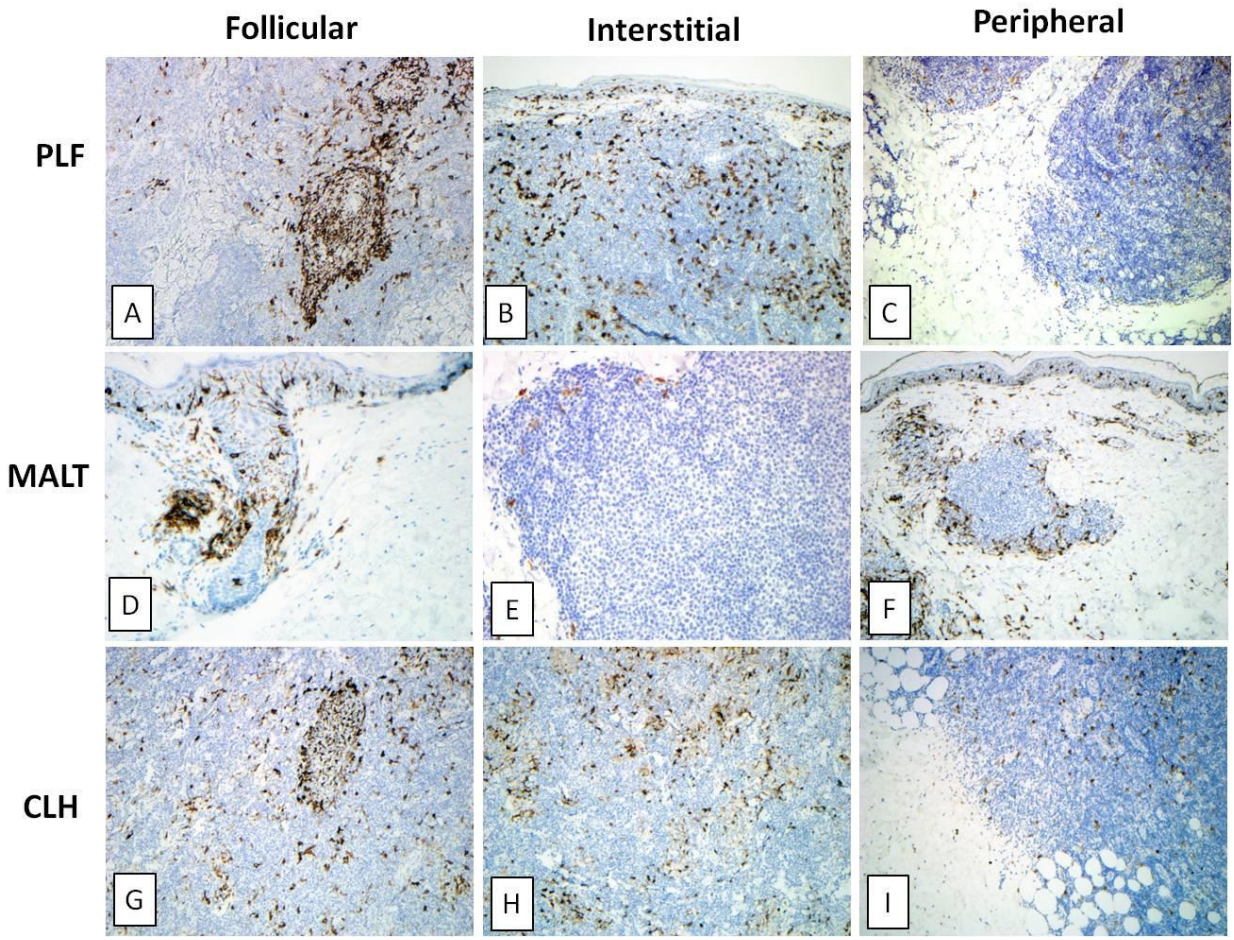
11.2 FIGURE 2

Percentage of cells staining positively for PD-1, CD3, and the ratio of PD-1/CD3 positive cells in pseudolymphomatous folliculitis (PLF), MALT lymphoma, and cutaneous lymphoid hyperplasia (CLH). Immunohistochemical staining reveals a statistically significant difference in the percentage of cells staining positively for PD-1 and the ratio of PD-1/CD3 positive cells among the three entities, but no significant difference in the percentage of cells staining positively for CD3.



11.3 FIGURE 3

CD1a in pseudolymphomatous folliculitis (PLF), extranodal marginal zone lymphoma of mucosa-associated lymphoid tissue (MALT lymphoma), and cutaneous lymphoid hyperplasia (CLH). Immunohistochemical staining patterns for CD1a staining were classified as follicular (within the hair follicle epithelium), interstitial (within the lymphocytic infiltrate), or peripheral (around the periphery of lymphoid nodules or excluded from reactive lymphoid follicles). In PLF v. CLH there are numerous CD1a+ dendritic cells are present within the hair follicle epithelium (A) and interspersed in the lymphocytic infiltrate (B). Peripheral staining is absent (C). MALT lymphoma with CD1a+ dendritic cells are present in the hair follicle epithelium (D). While interstitial CD1a+ dendritic cells are absent (E), there are numerous intensely-staining cells around the periphery of a lymphoid aggregate (F). CLH demonstrates numerous perifollicular (G) and interstitial (H) CD1a+ dendritic cells. Peripheral staining pattern is absent in CLH (I). (CD1a, 10x)


Follicular
Interstitial
Peripheral
PLF
MALT
CLH
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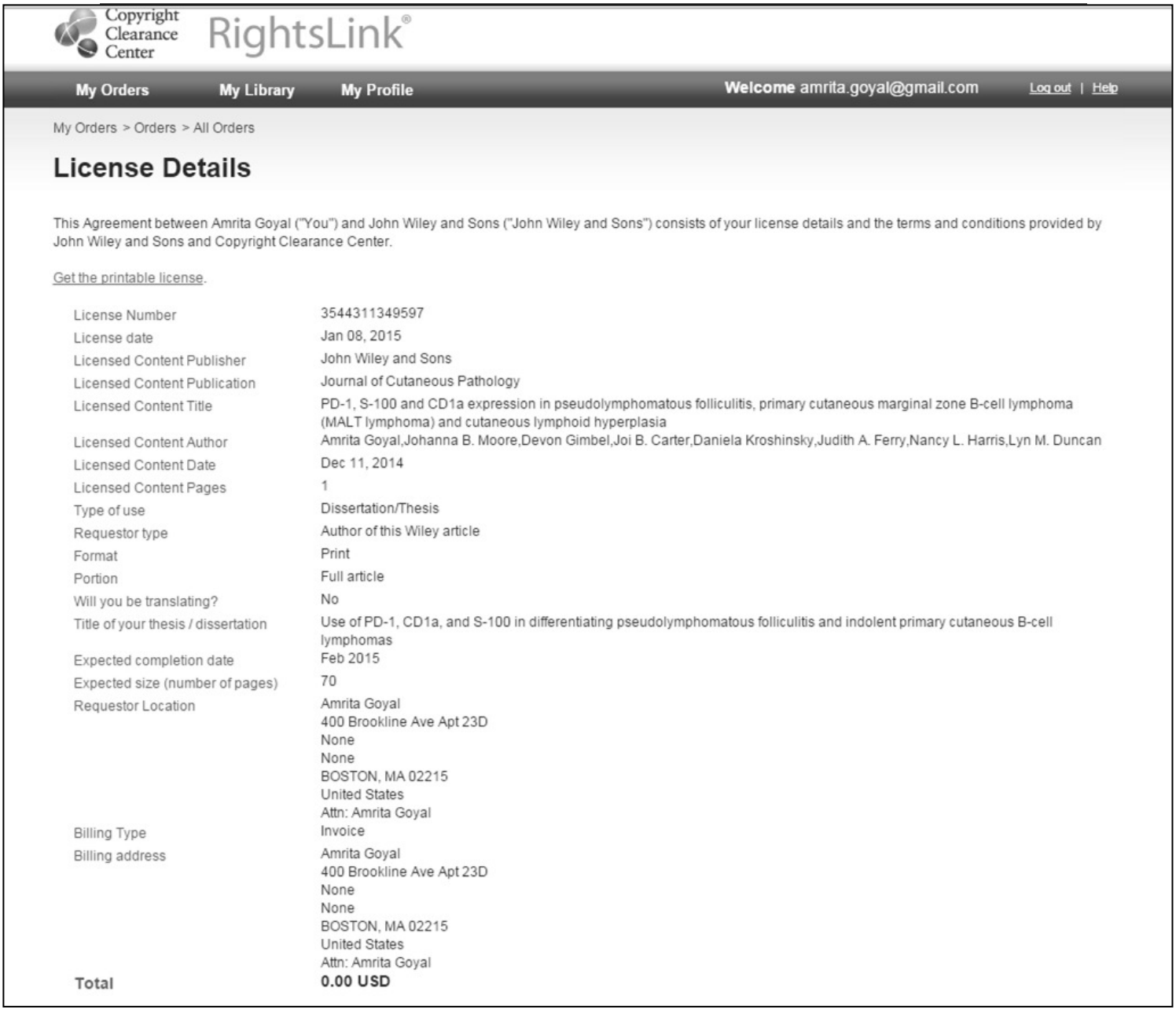

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