Absence of catagen/telogen phase and loss of cytokeratin 15 expression in hair follicles in lichen planopilaris

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Background: Lichen planopilaris (LPP) is a lymphocyte-mediated cicatricial alopecia mostly involving the bulge region of the hair follicle. The origin of LPP is unknown. Therapy for LPP often does not prevent disease progression. We describe histologic and immunohistologic features that aid in diagnosis and provide an explanation for disease progression in LPP.

Objective: We sought to demonstrate a decrease in the number of catagen-/telogen-phase follicles and to confirm the loss of cytokeratin 15 (CK15) expression in the stem cells of LPP-affected follicles.

Methods: In all, 144 LPP cases were retrieved; 55 cases were stained immunohistochemically, targeting the CK15 antigen with 40 cases ultimately analyzed for CK15 expression.

Results: Catagen/telogen phase was significantly decreased or absent in all cases of LPP, a novel clue useful in histologic diagnostics. The loss of $CK15^+$ stem cells in most affected follicles in LPP was also confirmed, with unaffected follicles retaining $CK15^+$ stem cells.

Limitations: Limited tissue for analysis remained in the clinical sample tissue blocks.

Conclusion: Damaged follicles that have lost their $CK15^+$ stem cells disappear when they enter catagen phase. $CK15^+$ stem cell loss explains the clinical observation that LPP progresses despite immunosuppressive therapies. Finally, the absence of catagen/telogen hair follicles is a helpful diagnostic clue for LPP. (J Am Acad Dermatol 2014;71:969-72.)

Key words: anagen; bulge; catagen; cytokeratin 15; follicular cycle; follicular stem cell; immunohistochemistry; infundibulo-isthmic; lichen planopilaris; telogen.

Lichen planopilaris (LPP) is a progressive cicatricial (primary scarring) alopetic process, which is difficult both for patients and clinicians to manage. Patients present on a spectrum, ranging from no symptoms to intense symptoms of itching, burning, or tingling of the scalp. The clinical signs of disease may be patchy or diffuse hair loss with perifollicular erythema and scale that hugs the base of the affected hair follicles. There is no one accepted cause, although immune-mediated and environmental causes are postulated.[1] Therapy with topical and oral anti-inflammatory and immunosuppressive agents is often required for long periods before control is attained and hair loss can progress despite intervention.

A definitive diagnosis of LPP is based on clinical and histopathological findings. Biopsy specimens show perifollicular and intrafollicular lymphocytes at the infundibulo-isthmic level with variable amount of blue-/gray-staining perifollicular fibrosis. Compound follicles, including the tufted type with multiple hair shafts, may form. A similar histologic pattern is seen in central centrifugal cicatricial

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alopecia and frontal fibrosing alopecia, the latter of which may be a variant of LPP.[2]

The lymphocytic attack is usually focused on the infundibulo-isthmic bulge portion of the follicle, where the cytokeratin 15 $(CK15)^+$ follicular stem cells reside. CK15 is an antigen marker expressed in human follicular bulge stem cells that has been shown to preferentially stain keratinocytes in the follicular bulge region.[3] A prior study demonstrated the loss of $CK15^+$ stems cells in affected follicles of LPP and other alopetic diseases in a small number of cases (7).[4] We confirm this prior demonstration in a much larger study of LPP and correlate this finding with the novel observation that catagen- and telogen-phase follicles are almost absent in cases of active LPP.

CAPSULE SUMMARY

- Lichen planopilaris targets the bulge stem cell region.
- We demonstrate the decrease and near absence of catagen-/telogen-phase follicles, a novel observation, and confirm the absence of cytokeratin 15^+ stem cells in follicles with active lichen planopilaris.
- Early diagnosis and treatment of lichen planopilaris may have an impact on disease progression.

METHODS

A total of 144 cases fulfilling the previously described clinical and histopathological criteria of LPP were studied. Cases were identified with a database search for a definitive diagnosis of LPP from archival files. All cases were patients of physicians (J. L. R. and N. D.) experienced in clinical interpretation of hair disorders. A clinical diagnosis of LPP was considered with or without frank alopetic patches, with or without positive hair pull. Catagen/telogen data were taken from the clinical pathology reports, which all contained a hair count table. Normal follicular density varies, but, in our experience, a normal density is between 1.6 to 3.2 follicles/mm^2.[5] Other clinical parameters investigated from the report included age, sex, and topographic location of the biopsy procedure. Of the 144 cases, 89 cases did not undergo further analysis, because the tissue blocks were unavailable (cases prior to 2010). The remaining 55 cases from 2010 to 2012 were analyzed histologically and immunohistochemically. The biopsy specimens (all referred from J. L. R. and N. D.) were 4-mm punch biopsy specimens, fixed in buffered formalin and transversely trisected using the method described by Frishberg et al.[6] The histopathological diagnosis of LPP was confirmed by the dermatopathologist (C. T. T.) who has experience in the histologic diagnosis of hair loss disorders. With the transverse sectioning technique of Frishberg et al,[6] the specimen is trisected, thereby allowing visualization of the follicular histology at 3 different levels. Level sections allow even more precise analysis of all levels of the follicle. The follicular bulge is known to reside below the sebaceous duct/lobule near the area where the arrector pili muscle attaches. In LPP, the focus of inflammation is between the lower infundibulum (identified by the granular layer) and the upper isthmus (no granular layer), and the bulge resides in this region.

To confirm the location of the bulge, $CK15^+$ epithelial cells were identified using immunohistochemistry. Antibodies targeting CK15 and nestin were tested on controls, because both have been reported to be specific to stem cells.[7-9] The normal controls used in assessing the CK15 and nestin antibodies were uninvolved follicles in LPP cases, because almost all cases of LPP have a mixture of involved and uninvolved (normal) follicles on histology. Given the nonspecific staining of the nestin antibody, it is possible that the antibody sold as one targeting nestin was not actually specific for this antigen. The nestin antibody was not used in the study. CK15 showed specificity for the cells in the bulge, though it also stained basal keratinocytes in the interadnexal epidermis. This basal expression correlates with prior work showing that the interfollicular epidermis is re-epithelialized with $CK15^+$ stem cells from the follicle after injury.[10] Interestingly, most of the cells in normal telogen-phase follicles (germs) are $CK15^+$. Of the 55 cases stained for CK15, 40 were analyzed because only these contained foci of active LPP.

RESULTS

Microscopic examination of all cases revealed histologic features characteristic to LPP. Briefly these included perifollicular fibrosis, compound follicles, and an infiltrate of lymphocytes centered around the infundibulo-isthmic region of the follicle (Fig 1). All 40 cases had a decreased and almost absent number of catagen-/telogen-phase follicles (Figs 2 and 3). Statistical analyses were performed using the Biostatistics and Design Program (Oregon Health and Science University, Portland, OR). For the 144 patients included in the sample, patients averaged 3.53% of follicles in catagen or telogen phase (95% confidence interval 2.58%-4.81%), which is

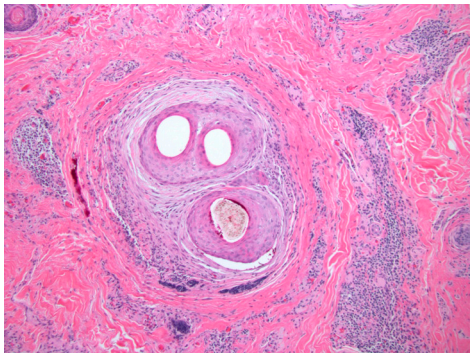

Fig 1. Lichen planopilaris. Perifollicular fibrosis and inflammation surrounding a compound follicle in lichen planopilaris.

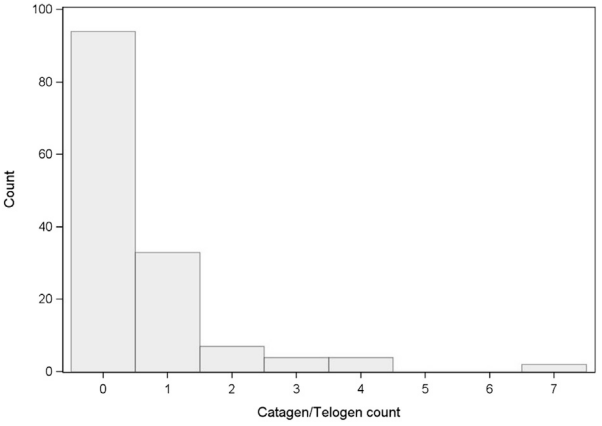

Fig 2. Distribution of catagen/telogen count in patients.

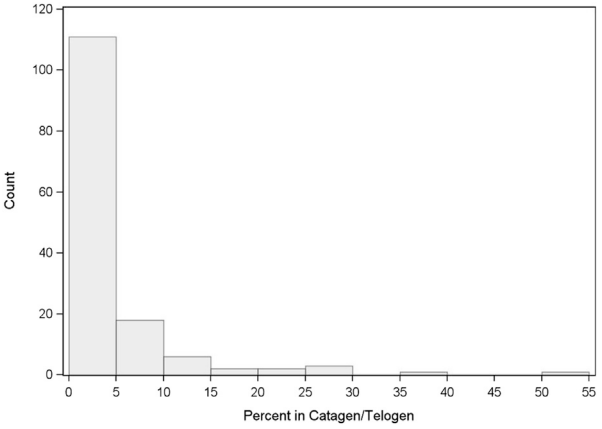

Fig 3. Percent distribution of catagen/telogen in patients.

significantly lower than the expected 8% to 10% in a healthy sample.

In 35 of 40 cases, CK15 immunohistochemical staining showed that in most follicles with perifollicular lymphocytes, CK15 expression was absent (Fig 4). A total of 89 involved follicles were identified in the 35 cases. Uninvolved follicles in the cases usually retained CK15 expression. In 5 of 40 cases, CK15 staining persisted in follicles involved with LPP.

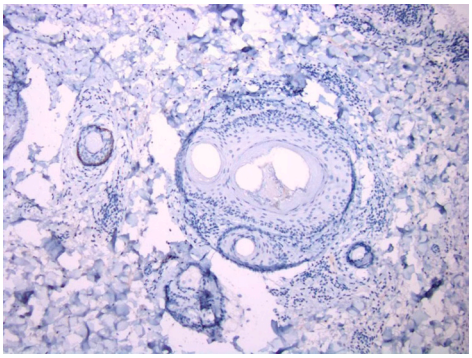

Fig 4. Lichen planopilaris. Loss of cytokeratin 15 expression.

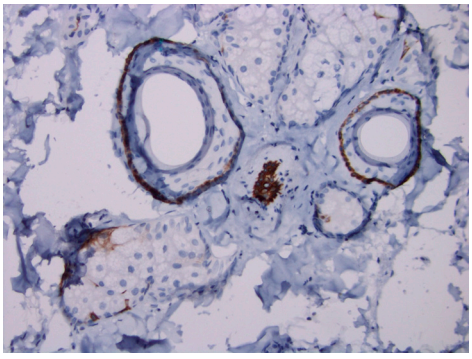

Fig 5. Lichen planopilaris. Retention of cytokeratin 15 expression in an uninvolved follicle.

Eight involved follicles were identified in the 5 cases (Fig 5). Importantly, the retention of $CK15^+$ stem cells in involved follicles was in those with minimal perifollicular fibrosis.

DISCUSSION

This study reports the novel observation that there is near absence of catagen- and telogen-phase follicle in active LPP. This study also confirms the previously reported finding that the focused infundibulo-isthmic lymphocytic inflammation in LPP destroys the $CK15^+$ stem cells in the bulge.[4]

Because scalp hair follicles have a long anagen phase (3-5 years) and grow in a dyssynchronous manner (cycling at different times), damaged follicles that have lost their $CK15^+$ stem cells will slowly disappear when they enter catagen phase. This explains the clinical observation that LPP and other cicatricial (primary scarring) alopetic diseases often progress despite immunosuppressive therapies. Importantly for the clinician, this study provides a strong argument that LPP and other cicatricial (primary scarring) alopetic diseases should be diagnosed and treated as early as possible. Future studies may show that the retention of $CK15^+$ stem cells in active cicatricial (primary scarring) processes is a useful prognostic tool. Based on the histologic

features of LPP, which usually has a focused infundibulo-isthmic location of the lymphocytes and the demonstration of $CK15^+$ stem cell loss, it is possible that the $CK15^+$ stem cells are an inflammatory or autoimmune target in LPP.

Future investigation of $CK15^+$ stem cells in LPP and other alopetic processes may not only help us understand the pathophysiology of these diseases but also better define the role of $CK15^+$ stem cells in the life of the follicle. Because of current limitations of hematoxylin-eosin and immunohistochemical analysis of the follicular cycle, future studies that better define the telogen phase could be useful in confirming our finding of loss of catagen/telogen phase in LPP.[11] The study is limited to cases of LPP, with other cicatricial alopecias not being included. In our experience, though, the same loss of catagen/telogen phase is not observed in processes such as folliculitis decalvans. Ongoing studies are being conducted to address this question.

Finally, for dermatopathologists, we show that the absence of catagen- and telogen-phase follicles is a regular histologic feature of LPP and that this feature is a useful diagnostic tool. Because the focused infundibulo-isthmic lymphocytic process in subtle LPP is sometimes difficult to identify on routine sections, the absence of catagen/telogen follicles when LPP is suspected should direct the pathologist to perform level sections.

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