Original Article

Immunohistochemical analyses of dermal cell infiltrates in Lichen Planopilaris: Relation to the pathogenesis of the scarring process

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Objectives: To investigate lesions from patients with LPP with regard to differences in the pattern of expression of some immuno-histochemical markers which may explain the pathogenesis of scarring process, which characterize this disease.
Methodology: Skin biopsies from twelve LPP patients were studied for cells expressing CD3, CD4, CD8, CD20, and CD68. They were stanied by double immunohistochemeistry means for the expression of cutaneous lymphocyte antigen (CLA) and the cytotoxic molecule, granzyme B.
Results: Double staining lesions revealed that the granzyme B-expressing lymphocytes were mostly of the CLA+ skin-homing phenotype, and the increased expression of granzyme B in LPP was associated histologically with the inflammatory infiltrate that involved the epidermis and the adnexal structures. The cellular infiltrate was demonstrated at the vicinity of the dermoepidermal junction and may be involved in the destruction of the overlying epidermis with secondary abnormal healing of the epidermis, secondary scarring and fibrosis.
Conclusions: This study provides an evidence of potentially autoreactive cytotoxic T-lymphocytes targeting adnexal structures in association with scarring LPP. It is, therefore, possible that these cells are responsible for scar formation. (Rawal Med J 2014;39: 150-153).
Key words: Lichen planopilaris, Inflammatory cell infiltrate, immunohistochemistry.

INTRODUCTION

Lichen planopilaris (LPP), a follicular form of lichen planus,[1] is a rare inflammatory lymphocyte mediated disorder that results in patchy progressive permanent hair loss mainly on the scalp.[2] It commonly develops in association with lichen planus affecting the skin, mucosa and nails. Although lichen planopilaris is rare, it is one of the common causes of scarring hair loss of the scalp. LPP histologically has been reported to show two different patterns.[3] In the first pattern, hair follicles and the perifollicular dermis are mainly involved, with no involvement of the interfollicular structures. In the second pattern, the pathologic changes extended to the interfollicular epidermis and the papillary dermis, the interfollicular changes were similar to those seen in LP of glabrous skin with hyperkeratosis, hypergranulosis of the follicular infundibula with the presence of surrounding lichenoid inflammatory infiltrates of lymphocytes.

Although the physiopathology is unclear, an autoimmune etiology is generally accepted.[4-6] Infiltrating T-lymphocytes are considered to play a major pathological role in skin lesions of LPP. The lymphocyte infiltrate is composed mainly of CD3+ (mature T) cells with few CD20+ (mature B) cells. CD8+ cells outnumbered CD4+ cells.[5,6] LPP is a scarring disease and little is known about the pathogenesis of the scarring process.[6,7] Work has been done previously on patients with discoid lupus erythematosus (DLE), another scarring disease[8] which provided evidence that autoreactive cytotoxic lymphocytes targeting adnexal structures are highly associated with scarring lupus erythematosus lesions and might be responsible for their scarring character. The aim in this study was to investigate skin lesions from patients with LPP by immunohistochemistry means in order to determine any differences in the pattern and the density of the cellular infiltrate that may explain the liability of the disease to scar formation.

The Aim and Objectives of the Study.

This can be achieved by:

1. Investigating skin biopsies from LPP patients for cells expressing CD3, CD4, CD8, CD20 and CD68.
2. Determining the cytotoxic function of T lymphocytes (CD8+) by investigating skin biopsies for the expression of cutaneous lymphocyte antigen (CLA) and the

cytotoxic molecule, granzyme B.

3. Determining whether the coexpression of the cytotoxic lymphocytes (CD8+ cells) also expressed CLA and whether the coexpression of the cytotoxic molecule, granzyme B was also expressed by CLA positive cells.

METHODOLOGY

Twelve patients with LPP (10 females, 2 males) with skin lesions were included in the study. They were compared with 7 normal controls (5 females, 2 males). Informed consent was obtained from all participants. The age of patients and controls ranged between 27 and 56 years. All patients underwent a skin biopsy from the edge of the most recent patch of the disease. Standard hematoxylin and eosin staining was performed. For the double immunohistochemistry, both the Novolink™ Polymer detection System and the Vectastain® ABC system were used for staining.

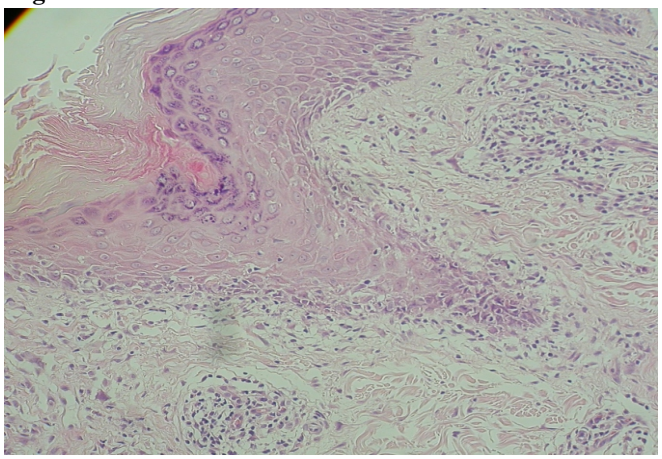
The biopsies were labeled with monoclonal antibodies to a variety of cell markers (CD3, CD4, CD8, CD20, CD68, CLA and granzyme B). Double immunohistochemistry was used to detect CD8 with CLA, and CLA with granzyme B. For each biopsy, staining with each antibody was repeated twice at five different levels of sectioning. Dermal cell infiltrate was counted per high power field (magnification 200) manually.

Test of normality (Kolmogorov-Smirrov) was applied and data were then analysed by one-way ANOVA. Individual groups were then compared using the Tukey-Kramer method for calculation of the minimum significance difference. A $p<0.05$ was considered statistically significant. Data were analysed using SPSS software.

RESULTS

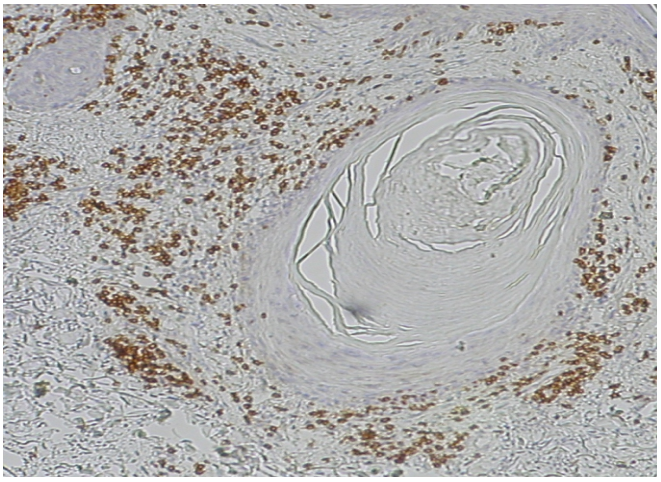
All biopsies showed typical features of a lichenoid reaction pattern (Figure 1) characterized by the combination of degeneration of the basal layer of the epidermis and a band like lymphocytic infiltrate obscuring the dermoepidermal junction. In addition, the inflammation was seen perifollicular with some involvement of the basal cell layers of the hair follicles which also showed basal cell degeneration.

Figure 1. LPP pathology showing a lichenoid reaction pattern characterised by the combination of degeneration of the basal layer of the epidermis and a band like lymphocytic infiltrate obscuring the dermoepidermal junction. The basal layer of the epidermis exhibits vacuolar degeneration.



The cytotoxic T cells were infiltrating the epidermis, especially in the vicinity of the dermoepidermal junction and perifollicular areas in LPP lesions. In addition, the entire hair follicle was involved in some cases with this inflammatory infiltrate and the whole hair follicle was obscured by the inflammatory process (Figure 2). Higher numbers of these cells were present in skin lesions of LPP lesions when compared with healthy controls ($P<0.01$). In addition, CD8+ cells outnumbered CD4+ cells in all examined biopsies sections.

Figure 2. CD3 positive cells in LPP patients. LPP lesion stained with CD3 monoclonal antibody, the CD3+ cells are mainly in perifollicular areas and the dermoepidermal junction.



The anti-CD68 monoclonal antibodies were very sparse in control biopsies. In LPP lesions, however, this CD68+ cells were significantly higher than in control biopsies; a large number of this cell infiltrate was present at the dermoepidermal junction of the follicular epithelium as well as the interfollicular epidermis.

The monoclonal anti-CLA was directed against CLA+ cells (CLA a specific skin homing receptor whose expression is up-regulated during transition of naïve to memory/effector T-cells). The expression of this antibody followed the pattern of the expression of other inflammatory cell markers. A higher number of CLA-expressing cells were present in skin lesions of LPP when compared with healthy control biopsies. Similar to the distribution of CLA positive cells was the Granzyme B; there was a strong expression of it at the dermoepidermal junction of the follicular epithelium as well as the interfollicular epidermis. The expression of CLA on CD8+ infiltrating T-lymphocytes in all of the biopsies from the patients with LPP was seen. The granzyme B-expressing lymphocytes were mostly of the CLA+ skin-homing phenotype.

DISCUSSION

The inflammatory process of LPP frequently results in the disruption of the basal cell of the external root sheath of the hair follicle and resulting in scar formation. Infiltrating T-lymphocytes are considered to play a major pathological role in skin lesions of LPP, but there have been very few studies[5,7] that have attempted to explain the development of scarring skin lesions. An alterations in the basement membrane zone in LPP that may explain the abnormal healing at follicular level which leads to irreversible hair loss and scarring.[6] Another explanation for the scar formation is that hair follicle stem cells which are vital for the growth of hair follicles and the adjacent epidermis showed abnormalities that might explain the irreversible scarring alopecia and scarring process in LPP.[6,7]

Discoid lupus erythematosus is another scarring disease and was investigated regarding the pathogenesis of scarring response. One of these studies showed that cytotoxic lymphocytes targeting adnexal structures were highly associated with scarring lesions.[8] The immunohistological data in this study confirmed earlier studies,[5,6] which were characterized by a dense lymphocytic infiltrate composed of CD3+ lymphocytes with suppressor/cytotoxic T-cells (CD8+) outnumbered the helper/inducer T-cells (CD4+). In present study, the predominant infiltrating cells were T-cells followed by macrophages (CD68+) then B-cells.

Macrophages are capable of antigen presentation and have a role in the regulation of immune responses and the development of inflammation.[9] Their distribution in lesions of LPP in this study with T-lymphocytes in the vicinity of the dermoepidermal junction and around appendages, may explain why there is damage to skin appendages and the overlying dermoepidermal junction with secondary fibrosis and scarring in LPP.

Granzyme B, a serine protease, is a potent toxin that can enter target cells and synergize with other granule toxins to cause cell death. This is an important mechanism by which the cytotoxic lymphocytes can protect their host from viral infection and cellular transformation by delivering a range of toxins stored within intracellular granules.[10] In our study, there was a strong expression of the cytotoxic molecule granzyme B specifically in lesional lymphocytes of patients with LPP. Granzyme B and CLA+ cells were dense mainly peri-adnexal. A large number of these cells were present at the dermoepidermal junction of the follicular epithelium as well as the interfollicular epidermis. There is a need to demonstrate these changes in the expression of inflammatory markers in non scarring glabrous type of LP. Limitations of the Study include limited number of the cases due to the rarity of the disease.

CONCLUSIONS

The cellular infiltrate was demonstrated at the vicinity of the dermoepidermal junction and may be involved in the destruction of the overlying epidermis with secondary abnormal healing of the epidermis, secondary scarring and fibrosis in patients of Lichen planopilaris.

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