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Hyperkeratosis Lenticularis Perstans (Flegel's Disease)

In Situ Characterization of T Cell Subsets and Langerhans' Cells

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We report a patient with hyperkeratosis lenticularis perstans (HLP) manifesting as multiple reddish-brown hyperkeratotic papules on the lower extremities. Typical histologic features of HLP include hyperkeratosis, thinning or absence of the granular layer and a band-like infiltrate in the upper dermis underlying an atrophic epidermis. In order to determine the cellular composition of the infiltrate, skin biopsy specimens were studied immunohistochemically using a series of commercially available monoclonal antibodies. The dermal infiltrate consists predominantly of helper/inducer T cells (Leu-4+, Leu-3a+). Suppressor/cytotoxic T cells (Leu-2a+) were fewer at the periphery of the infiltrate. The majority of T cells were activated as they expressed HLA-DR-antigen. Large numbers of Leu-6+ Langerhans' cells were observed at the dermo-epidermal interface. Few natural killer cells (Leu-11b+) were noted within the dermal infiltrate. These findings support the hypothesis that an active cellular immune reaction involving the epidermis is of pathogenic importance for HLP. *Key words: Immunohistochemical staining; Monoclonal antibodies.* (Received October 10, 1987.)

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Hyperkeratosis lenticularis perstans (HLP) is a rare dermatosis, first described in 1958 by Flegel (1). This disorder of keratinization appears to be transmitted in autosomal dominant fashion (2, 3). Flegel's disease is characterized clinically by asymptomatic multiple pink to reddish-brown hyperkeratotic papules occurring most frequently over the dorsa of the feet and the lower parts of the legs and arms (1, 4–13). The typical histopathologic findings are hyperkeratosis with occasional areas of parakeratosis, atrophy of underlying epidermis and a well-circumscribed band-like infiltrate in the upper dermis (1, 4, 5, 10).

In this paper, we describe the application of monoclonal antibodies for T cell subsets and Langerhans' cells and the use of an indirect immunoperoxidase technique to characterize the inflammatory infiltrate in well developed lesions of HLP.

MATERIAL AND METHODS

A skin biopsy was taken from the lesion on the lower part of the leg. One portion of the sample was frozen immediately in liquid nitrogen and the remainder submitted for routine light microscopy study using hematoxylin-eosin staining. Cryostat sections were made at 6 μm and mounted on slides that had been coated with 0.2% gelatin solution and dried at room temperature.

The monoclonal antibodies (Becton Dickinson, Sunnyvale, Calif., USA) employed in this study and their specificities were as follows:

Anti-Leu-4	Pan T cell
Anti-Leu-2a	cytotoxic/suppressor T cell
Anti-Leu-3a	helper/inducer T cell
Anti-Leu-6	Langerhans' cell

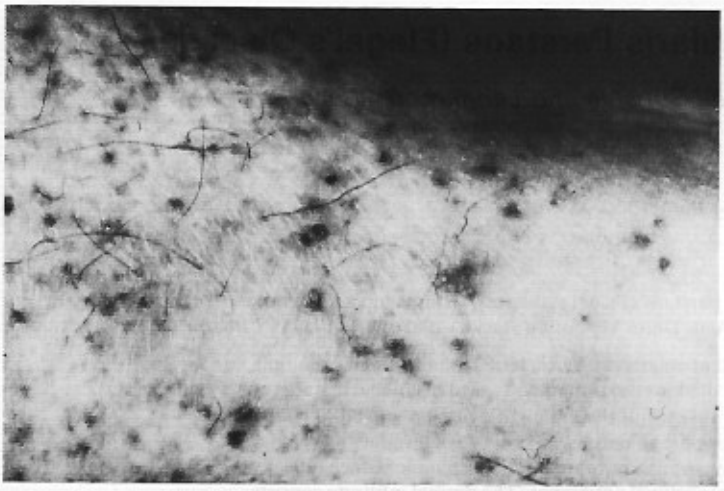


Fig. 1. Hyperkeratosis lenticularis perstans. Hyperkeratotic papules on the lower extremities.

Anti-Leu-14	B cell
Anti-Leu-11B	natural killer cells
Anti-HLA-DR	activated T cells, Langerhans' cells, keratinocytes
Anti-IL-2	interleukin-2-receptor

Cryostat sections were fixed in acetone for 10 min at 4°C. Sections were initially incubated with phosphate-buffered saline (PBS) for 3 min at 20°C. Primary antibodies, diluted 1 : 30, were placed on the sections for 15 min. After washing and incubation with PBS for 10 min, the goat anti-mouse antibody, peroxidase-conjugated (second antibody), was placed on the sections at a dilution of 1 : 20 for 15 min. After washing with PBS, sections were stained for peroxidase for 15 min in a solution containing 10 mg of 3-amino-9 ethyl-carbazole dissolved in 2.5 ml of *N,N*-dimethylformamide, 47.5 ml of 0.17 M acetate buffer at a pH of 5, 12 and 0.5 ml of 3% H_2O_2. Counterstaining was done with hematoxylin and mounting was carried out with glycerine jelly.

In addition, cryostat sections were stained for the presence of acid phosphatase (14) and α-naphthyl acetate esterase (non-specific esterase).

Case report

A 49-year-old woman presented with a 5-year history of mildly pruritic, hyperkeratotic papules on the lower extremities. The patient's father was also found to have an identical eruption on the lower legs.

On physical examination there were multiple pink to reddish-brown hyperkeratotic papules 1–5 mm in diameter involving the dorsa of the feet and the anterior and posterior aspects of both legs. Prominent lesions were slightly depressed in their centres (Fig. 1). The horny papules could be removed in one piece, leaving a slight bleeding.

The patient was given isotretinoin (Roaccutan®) in a dose of 20 mg daily. Clinical improvement of the skin lesions was noted after 3 weeks of treatment. During the subsequent 2 months the patient was treated with etretin in a dose of 20 mg daily, with dramatic improvement of the skin lesions.

Histopathological findings

The hematoxylin and eosin stained sections showed a well defined area of massive, compact and lamellar hyperkeratosis with occasional areas of parakeratosis overlying an atrophic epidermis. There was a very sharp transition from the flattened to normal epidermis. A band-like infiltrate with sharp demarcation at its lower border was closely attached to the lower surface of the epidermis and strictly limited to the papillary dermis underlying the lesion.

At each lateral margin of the infiltrate, rete ridges tended to extend downward. The infiltrate was composed predominantly of lymphocytes. The basal cell layer was flattened and showed occasional degeneration of the basal keratinocytes. No exocytosis of lymphocytes was observed (Fig. 2).

Immunohistochemical findings

Our study shows that the vast majority of mononuclear cells in the dermal infiltrate stained with Leu-4 antibodies, providing evidence that they are T lymphocytes (Fig. 3). Most of these Leu-4 positive cells

Fig. 2. Hyperkeratosis lenticularis perstans. Heavy hyperkeratosis and a band-like infiltrate underlying an atrophic epidermis (HE, ×24).

also reacted with Leu-3 antibodies and, therefore, were of the helper/inducer phenotype. Leu-2a reactive suppressor/cytotoxic T cells were fewer at the periphery of the infiltrate.

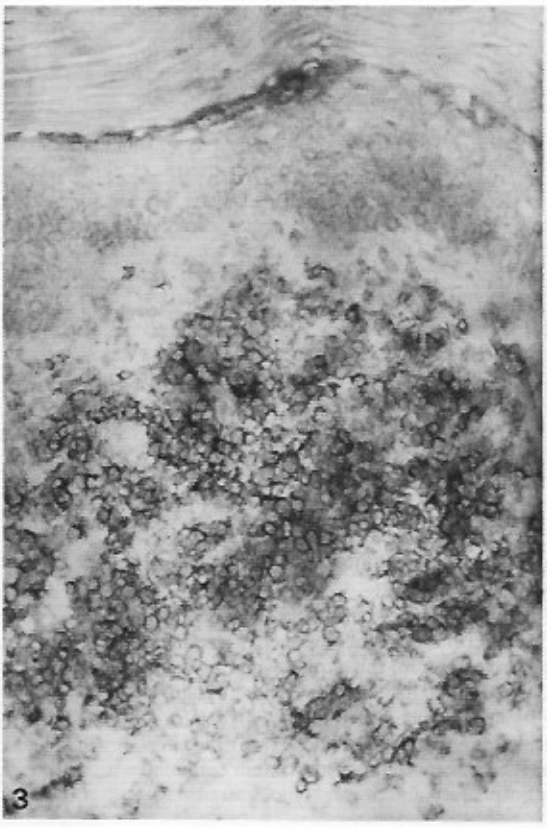
Staining with Leu-6 antibody revealed large numbers of dendritic Langerhans' cells at the dermo-epidermal interface and within the dermal infiltrate (Fig. 4). Only occasional Leu-6 reactive dendritic Langerhans' cells were found in the atrophic epidermis. Large proportions of the dermal lymphoid cells were HLA-DR positive T cells, i.e. activated T cells. No HLA-DR-antigen stained epidermal keratinocytes were seen, although few Langerhans' cells were clearly stained. Very few Leu-11B reactive natural killer cells were observed within the dermal infiltrate. Cells bearing the interleukin 2 (IL-2) receptor were present only in very small numbers. No Leu-14 positive B cells were found. The stratum corneum of HLP demonstrated a very strong diffuse activity of acid phosphatase and non-specific esterase.

DISCUSSION

Hyperkeratosis lenticularis perstans is a rare disorder of keratinization presenting with multiple hyperkeratotic papules (1, 6–13). The lower extremities are the usual site of involvement, although rarely lesions have occurred on the arms, hands, trunk, palms and soles and even on the oral mucosa (5, 10, 13). The condition is reported to affect people in mid to late life and it seems to be transmitted in an autosomal dominant fashion (2, 3, 5, 10).

Histologically the predominant features are prominent hyperkeratosis overlying an atrophic epidermis and a band-like infiltrate in the papillary dermis composed predominantly of lymphocytes (1, 3, 5, 9, 10). The granular layer is usually of reduced thickness or even absent. The characteristic ultrastructural finding reported in the literature is a decrease in or complete absence of lamellar (Odland) bodies in the epidermis (5, 12, 15–17). In contrast, other authors reported the presence of coating granules in lesional skin of HLP (8, 11). It has been reported by several authors that the lack of Odland bodies in the epidermis may play an important role in the pathogenesis of Flegel's disease (5, 10, 17).

On the contrary, Flegel (1) considered the inflammatory changes in the papillary dermis to be one of the primary event occurring in the earliest lesion of HLP. We suggest that the inflammatory components are intimately associated with the early events in the pathogenesis of the HLP and that they may not be dismissed as a secondary phenomenon. In the present case, the results of the immunohistological study employing monoclonal antibodies showed that the majority of the dermal infiltrate are helper/inducer T cells. Staining with Leu-6 antibody revealed a considerable number of Langerhans' cells in the papillary dermis and at the dermo-epidermal interface. HLA-DR-antigen was demonstrated on the vast majority of



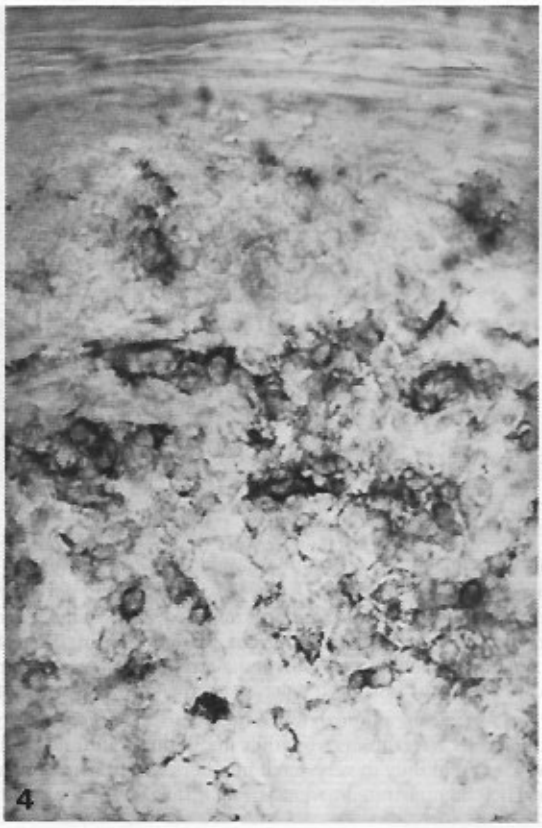


Fig. 3. Hyperkeratosis lenticularis perstans. The dermal infiltrate consists almost exclusively of Leu-4 reactive T cells (Immun-POX, ×70).

Fig. 4. Hyperkeratosis lenticularis perstans. Leu-6 reactive dendritic Langerhans' cells within the infiltrate in the upper dermis (Immun-POX, ×105).

cells in the infiltrate. HLA-DR-antigen has been thought to be necessary for the initiation of a cellular immune reaction (18).

Although we were unable to demonstrate the presence of exocytic T cells, other investigators, using electron microscopy, have reported that some lymphocytes are present in the intercellular spaces of the lower epidermis associated with vacuolization of the basal keratinocytes (17). Activation of helper/inducer T cells gives rise to substances that stimulate various cell types, including macrophages, Langerhans' cells and keratinocytes (19). Recent immunohistochemical studies using monoclonal antikeratin antibodies support the concept that there is a hyperproliferation of keratinocytes in early lesions of HLP (10). The presence of large numbers of Langerhans' cells and macrophages at the dermo-epidermal interface may possibly indicate a primary role for T cells and Langerhans' cells in the pathogenesis of Flegel's disease.

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