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Lichen planus is associated with human herpesvirus type 7 replication and infiltration of plasmacytoid dendritic cells

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Summary

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Background Lichen planus (LP) is a common inflammatory skin disease of unknown aetiology. Viral causes have been suggested.

Objectives To find candidate viruses associated with LP.

Methods Lesional and nonlesional skin samples, peripheral blood mononuclear cells and serum were obtained from patients with LP. Ultrastructural, viral DNA, immunohistochemical and serological analyses were performed, and comparisons were made with psoriatic and normal skin.

Results Electron microscopy revealed typical 120–200-nm enveloped particles with a 100-nm nucleus resembling human herpesvirus (HHV) virions both in dermis and in epidermis of lesional LP tissue. HHV-7 DNA was found in 11 of 18 lesional LP samples, as opposed to only one of 11 nonlesional LP samples ($P = 0.06$), two of 11 lesional psoriasis samples ($P = 0.05$) and none of four normal skin samples. No relation was found between LP skin and DNA of other known HHVs (HHV-1–6 and 8). With immunohistochemistry, significantly more HHV-7+ cells were found in lesional LP epidermis than in normal epidermis. Lesional LP dermis contained significantly more HHV-7+ cells than nonlesional LP, psoriatic or normal dermis. Moreover, LP skin contained overwhelmingly and consistently more plasmacytoid dendritic cells (upregulated in virally induced conditions) than nonlesional LP samples.

Conclusions We conclude that HHV-7 replicates in LP lesions, but not in psoriasis, another inflammatory skin condition. HHV-7 is possibly involved in the pathogenesis of LP. These preliminary data make further research on this topic of interest.

Lichen planus (LP) is an inflammatory skin disease with a self-limiting course.^{1,2} The aetiology is unknown but it is speculated that LP is caused by a viral infection.³ Hepatitis C virus (HCV) has been suggested as a causal agent for LP,⁴ but epidemiological correlations are not strong.⁵ Nevertheless, viral causality in LP seems plausible because of the acute onset and the overall single episode presentation.

The goal of this study was to explore a possible association of viruses with LP, based on ultrastructural analysis of LP skin that revealed human herpesvirus (HHV)-like particles.

Materials and methods

Patients were selected on the basis of typical LP-like lesions after histopathological confirmation. Before treatment, full-

thickness skin biopsies were obtained from lesional and nonlesional skin and processed for routine histopathology, electron microscopy, DNA analysis and immunohistochemistry. Heparinized peripheral blood and serum were collected. Controls consisted of normal skin from breast reduction residual tissue, and lesional and nonlesional skin of patients with psoriasis. In all patients the herpes simplex virus (HSV), varicella-zoster virus (VZV), Epstein–Barr virus (EBV), cytomegalovirus (CMV), hepatitis A virus (HAV), hepatitis B virus (HBV) and HCV serostatus was determined.

DNA isolation and the nested polymerase chain reaction (PCR) for open reading frame 65 of HHV-8 have previously been described.⁶ The sense primer HHV6A (5'-TGAGGGTT-TGTTGGCTTTCTTAT-3') and the antisense HHV6B (5'-AGATG-GATAAGAAATGCGAG-3') were used in the first PCR of HHV-6

and the sense HHV7-1 (5'-TTACATGCAGATGACATTCTCA-3') and antisense HHV7-2 (5'-TGGATTGACACGCGGTGATT-3') were used in the first HHV-7 reaction. One-twentieth of the amount used in the first reaction was subjected to the second round of amplification by using the sense primer HHV6C (5'-GACGAGTATTTGAATTTCCGC-3') and antisense HHV6D (5'-TGGATGGTAGTTGATTAAGTTGAG-3') for HHV-6

detection and the sense primer HHV7-3 (5'-ATGTTGGACC-TGTAACGCACA-3') and antisense HHV7-4 (5'-ACTGTTGATTACGTTGACAG-3') for HHV-7 detection. The amplified fragments (257 bp for HHV-6 and 296 bp for HHV-7) were identified by gel electrophoresis. The lower limit of detection of these PCRs is 1–5 copies of HHV-6/7/8 DNA. Biopsies and peripheral blood mononuclear cells (PBMC) were screened for HSV-1, HSV-2, VZV, EBV and CMV using nested type-specific PCR against viral antigens as described before.⁷

The monoclonal antibodies used in this study were directed against: HHV-7 tegument protein pp85 (Advanced Biotechnologies Inc., Columbia, MD, U.S.A.), CD4, CD8 and CD123 (all from BD Biosciences, San Jose, CA, U.S.A.) and von Willebrandt factor (Dako, Glostrup, Denmark). Immunohistochemical staining was performed in triplicate as described before.⁸ HHV-7 expression was assessed at $\times 200$ magnification using a light microscope with an ocular grid, and expressed as number of positive cells mm^{-2} .

We used the paired samples t-test for inpatient comparisons, the independent samples t-test for interpatient comparison and the McNemar test for binominal data. $P \leq 0.05$ was considered significant.

Table 1 Nested polymerase chain reaction detection of herpesvirus (HHV) DNA in lichen planus, psoriasis, normal skin and peripheral blood mononuclear cells (PBMC).

	HHV-7	HHV-6
Lichen planus		
Lesional	11/18 (61%)* [#]	0/18
Nonlesional	1/11 (9%)*	0/11
PBMC	5/13 (38%)	2/13 (15%)
Psoriasis		
Lesional	2/11 (18%) [#]	1/11 (9%)
Nonlesional	0/3	0/3
Normal skin (healthy donors)	0/4	0/4

* $P = 0.06$; [#] $P = 0.05$ (McNemar test).

Fig 1 Immunohistochemical images of lesional (a,c,e,f) and nonlesional (b,d) lichen planus skin. (a) Human herpesvirus (HHV)-7+ cells in lesional and (b) nonlesional skin (insets: higher magnification of HHV-7+ cells). (c) CD123+ plasmacytoid dendritic cells in a band-like configuration close to the basal cell layer in lesional skin. (d) Few CD123+ cells in nonlesional skin. (e) CD4+ and (f) CD8+ lymphocytes located in lesional lichen planus skin (peroxidase staining; scale bar = 200 μm).

Fig 2. Mean $\pm$ SD cell count mm^{-2} of immunohistochemically positive human herpesvirus-7 cells in the epidermis and dermis in psoriatic skin (striped bars), lesional (black bars) and nonlesional (grey bars) skin from patients with lichen planus and in normal skin (white bars) from healthy volunteers. * $P < 0.05$ with paired samples t-test; # $P < 0.05$ with independent samples t-test.

Results

Eighteen patients were included. Their mean $\pm$ SD age was 43.9 ± 11.5 years, and the mean $\pm$ SD disease duration was 4.7 ± 3.3 months. The study was approved by the hospital ethics committee.

In all lesional samples but not in the nonlesional samples of four patients, herpesvirus-like particles were seen in cells of the epidermis and upper dermis. These particles consisted of 100–110-nm spiked spherical nucleocapsids surrounded by an asymmetrical 120–200-nm tegument.

One patient had a chronic active HBV infection, and one an HCV infection. Seven patients had previous infection with CMV, and six with HAV. All patients had previous VZV and EBV infections.

All samples were free of HSV-1, HSV-2, VZV, CMV and HHV-8 DNA. EBV DNA was detected in two of 15 LP lesional samples. HHV-7 DNA was detected in 11 of 18 (61%) lesional LP samples compared with one of 11 nonlesional LP biopsies (9%, $P = 0.06$) and two of 11 psoriasis biopsies (18%, $P = 0.05$, Table 1). Five of 13 PBMC samples from patients with LP were HHV-7 DNA positive (38%), and two were HHV-6 DNA positive (15%). One psoriasis biopsy was HHV-6 DNA positive (9%). None of the three nonlesional psoriasis biopsies and none of the four normal skin samples contained either HHV-6 or HHV-7 DNA.

In the epidermis and upper dermis of lesional LP tissue considerably more HHV-7+ cells were present (Fig. 1a) than in nonlesional LP tissue (Fig. 1b). Lesional LP dermis contained significantly more HHV-7+ cells than nonlesional, psoriatic or normal dermis (Fig. 2). Lesional LP epidermis contained significantly more HHV-7+ cells than normal epidermis.

Consistently in five patients, large numbers of CD123+ plasmacytoid dendritic cells (PDCs) were detected in a band-like lymphocytic configuration close to the basal layer in lesional LP dermis (Fig. 1c), whereas in nonlesional LP skin and in normal skin samples, only sporadic CD123 expression was

detected (Fig. 1d). PDCs were present in the same location as CD4+ and CD8+ cells (Fig. 1e,f).

Discussion

At an ultrastructural level, herpesvirus-like particles were found in lesional LP tissue. We subsequently tried to identify the herpesvirus involved. No HSV-1, HSV-2, CMV, VZV or HHV-8 DNA was detectable in LP skin. EBV DNA was identified in two of 15 lesional LP samples, but none of these patients had serological signs of an active EBV infection, which makes EBV unlikely to be a possible trigger in LP pathogenesis.

HHV-7 DNA was detected in 11 of 18 lesional LP samples, in contrast to only one of 11 nonlesional samples. At $P = 0.06$ the significance criterion was not met, but we attribute this to the low number of samples. Psoriasis skin was included as control because, like LP, it is an inflammatory skin disease characterized by lymphocytic involvement. Only two of 11 psoriatic skin samples contained HHV-7 DNA. With the absence of HHV-7 DNA in the other controls this supports our idea that HHV-7 replication occurs specifically in LP.

HHV-7 antigen was detected significantly more often in lesional LP epidermal cells than in epidermal cells of healthy volunteers. Lesional dermis from patients with LP contained significantly more HHV-7+ cells than dermis from control groups, indicating that the HHV-7 infection is mainly located in the dermis.

HHV-7 has a high seroprevalence worldwide⁹ and is acquired by 90% of the population during childhood.^{10,11} The presence of HHV-7 in lesional LP skin does not directly imply a causative relation between the two. Viruses can replicate in inflammatory processes without any contributing effect. For this reason we tried to find further support for a viral cause for LP and characterized the inflammatory infiltrate in this disease.^{12,13} We found abundant numbers of CD123+ PDCs in lesional LP tissue, in close approximation to the basal epidermal layer, similar to the typical LP band-like lymphocytic infiltration. There was far less or no PDC accumulation in nonlesional LP tissue, psoriatic skin or normal skin. These preliminary data make further research on this topic of interest.

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