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RESEARCH ARTICLE

Human papilloma virus-16-specific CD8+ T-cell expansions characterize different clinical forms of lichen planus and not lichen sclerosus et atrophicus

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Abstract

Lichen planus (LP) is a cutaneomucosal chronic inflammatory disease characterized by a $CD8^+$ cytotoxic T-lymphocytes (CTL) infiltrate. In erosive oral LP, we found HPV16-specific activated CTL in lesions, supporting a pathogenic contribution of HPV16. Here, we investigated whether a similar scenario occurs in other clinical forms of LP and in lichen sclerosus et atrophicus (LSA), another chronic disease also affecting the mucosa and/or the skin. Blood CTL from LP and LSA patients expressed significant higher levels of granzyme B, perforin and CD107a proteins than healthy donors. Expansions of $TCRVß3^+$ CTL, with presence of TCR clonotypes identical to those previously detected in erosive oral LP, were found both in blood and mucosal/skin lesions of LP, and not of LSA patients. These expansions were enriched with HPV16-specific $CD8^+$ T-cells as shown by their recognition of the $E7_{11\text{-}20}$ immunodominant epitope. In LSA patients, the peripheral repertoire of CTL was oligoclonal for $TCRVß6^+$ CTL. Finally, although patients with LP and LSA have developed antibodies against HPV16

Marie-Lise Gougeon and Nicolas Fazilleau have contributed equally to this work.

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capsid L1, antibodies against HPV16 E6 were only observed in patients with LP. Overall, our data collectively suggest an involvement of HPV16-specific CTL in different clinical forms of LP, not only in erosive oral LP, while a different scenario operates in LSA.

KEYWORDS
cytotoxic T lymphocytes, human papilloma virus, lichen planus, T-cell, T-cell receptor

1 | INTRODUCTION

Lichen planus (LP) is a chronic inflammatory disease of unknown aetiology, involving the skin and mucous membranes, characterized by an infiltrate of immune cells, mainly $CD8^+$ cytotoxic T-lymphocytes (CTL), associated with epithelial cell death and disruption of basement membrane zone, as pyramidal features.[1] Numerous clinical and evolutive forms are described, as well as different locations (palms, soles, hair, nails, oral, genitals),[2] all sharing the same pathophysiological features with a cell-mediated immune damage of the basal keratinocytes potentially through the recognition of foreign antigens. Analysis of LP skin biopsies indicated involvement of type I IFNs, associated with the recruitment of $CXCR3^+$ and granzyme B^+ lymphocytes, indicating a Th1-biased cytotoxic immune response.[3,4]

Several triggering factors have been advocated in the mechanistic scenario in LP, including drugs, metals, bacterial or viral antigens.[5,6] Among viral candidates, the association of LP with hepatitis B virus (HBV) infection, suspected as soon as three decades ago[7] and reinforced by the description of LP onset after HBV vaccination,[8,9] was ultimately ruled out by several large case–control studies conducted in different countries with high HBV prevalence.[10–12] For hepatitis C virus (HCV) infection,[13] several meta-analyses demonstrated a significant association between HCV infection and oral LP,[14–16] with clinical benefit of HCV antiviral drugs on LP.[17] More recently, viral species with known mucosal and/or skin tropism, such as Epstein–Barr virus (EBV), *Herpes simplex* virus and Human papillomavirus virus (HPV), attracted special interest.[18] Regarding HPV, meta-analysis of published case–control studies showed that patients with oral LP had a significantly higher HPV detection in oral mucosa, by either PCR, immunohistochemical staining or in situ hybridization methods, than healthy controls, with a strong association with HPV16 genotype and higher rates of detection in erosive versus non-erosive oral LP lesions.[19–21]

Altogether, these epidemiological associations, as well as the T-cell signature of LP, raised the hypothesis of a mechanistic link bridging HPV-mucosal and cutaneous infection and cytotoxic T-cell responses in LP. Tackling this hypothesis, we recently showed that patients with erosive oral LP share oligoclonal expansions of HPV16-specific CTL in both the peripheral blood and the epithelial lesions, suggesting a key contribution of HPV16-specific CTL to the pathogenesis of erosive oral LP.[22] Here, we address the question of whether this immunological pattern is specific to erosive oral LP, or is also observed in other clinical forms of LP, and in a dermatosis also affecting the genital mucosa, the lichen sclerosus et atrophicus (LSA).

2 | METHODS

2.1 | Patients and healthy donors

Ten patients with LP, excluding patients with erosive oral LP and 10 with LSA were enrolled, at a time when skin or mucosal lesions were flaring. The Institutional Review Board (Comité de Protection des Personnes Ile de France IV, n°IRB 00003835) approved the study (EUDRACT n°2015-A01697-42). The patients were fully informed and gave a written consent for blood and skin collections. For all of them, blood and a viral skin swab were collected. Skin biopsies of LP and LSA lesions for research purpose were optional and realized in a subset of willing patients as indicated in Table S1. Healthy donors (HD) were blood donors from the French National Blood Service, seven were analysed in flow cytometry and 20, sex- and age-matched to the 10 LP and 10 LSA patients, were analysed for molecular biology studies. All the studied patients had negative serologies against HIV, HCV or HBV infection. The study was approved by the French South-West & Overseas ethical committee and was registered at the French Ministry of Higher Education and Research (DC-2015-2488). All experiments were performed in agreement with the guidelines of the Declaration of Helsinki.

2.2 | Antibodies and flow cytometry analysis

Fresh blood cells were stained with anti-CD3-PerCP-Cy™5.5 (Clone UCHT1, BD Biosciences), anti-CD45-APC-H7 (2D1, BD Biosciences), anti-CD8-V500 (SK1, BD Biosciences), Dextramer-HPV16 E711-20-PE (Dext-HPV PE; HLA-A*0201; YMLDLQPETT, Immudex), Dextramer-HIV-1 P17 Gag 77-85-PE (Dext-HIV-PE; HLA-A*0201; SLYNTVATL, Immudex), anti-TCR-Vß3-FITC (JOVI-3, Ancell), anti-HLA-A2 PE-Cy7 (BB7.2, BD Biosciences), anti-Perforin PE-CF594 (δG9, BD Biosciences), anti-Granzyme B BV421 (GB11, BD Biosciences), anti-CD107a-APC (H4A3, BD Biosciences) were used. Cells were incubated with dextramer for 10 min at room temperature, then antibodies were added for 30 min at 4°C. Stained cells were analysed using a LSR Fortessa flow cytometer (BD Biosciences).

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2.3 | Purification of T-lymphocyte subsets

Dead cells were removed using the MACS® Dead Cell Removal kit. T-cells were isolated using anti-CD8 or anti-CD4 Ab-coated immunomagnetic beads (Miltenyi Biotec). Cell purities exceeded 95%. $CD3^+CD8^+TCRV\beta3^+$Dext-HPV16$^+$ or Dext-HPV16$^-$ populations were isolated using a MoFlo® Astrios™cytometer cell sorter (Beckman Coulter).

2.4 | T-Cell Receptor Repertoire Analysis

RNA was extracted using RNAeasy kit (Qiagen) and reverse-transcribed into cDNA using Superscript II (Invitrogen Life Technologies). Quantitative PCR amplifications were performed with TaqMan Universal PCR Master Mix (Applied Biosystems), TCRVβ gene segments specific oligonucleotides, TCRCβ-specific antisense primer and a fluorescent probe specific for the TCRCβ gene segment.[23] All TCRVβ gene segments were amplified in all studied cell populations. The qPCR products were further used for the amplification of dye-labelled oligonucleotides specific for Cβ and allowed to assess the complementary determining region 3 (CDR3) length distribution.

2.5 | Cloning and sequencing of CDR3β rearrangements

Vβ-Jβ PCR products were cloned in pCR®4Blunt TOPO vector (Invitrogen Life Technologies). Sequencing reactions were performed using the ABI PRISM Big Dye Terminator Reaction Kit (Applied Biosystem). Reaction products were analysed on an ABI 3130XL 16 capillaries (Applied Biosystems).

2.6 | Detection of Human Papillomavirus infection

Skin swabs were obtained from LP and LSA lesions using a cyto-brush (DNAPAP Cervical Sampler; Qiagen). The brush was then placed in 1 mL of Cervical Specimen Transport Medium (Digene). DNA was extracted with the QIAamp DNA minikit (Qiagen) according to the manufacturer's instructions. The presence of alpha, beta and gamma HPV DNA was detected by a type-specific multiplex genotyping assay, which combines multiplex PCR and hybridization to type-specific oligonucleotide probes on fluorescent beads (Luminex corporation), as described previously.[24–26] After PCR amplification, each reaction mixture was analysed by multiplex HPV genotyping using Luminex technology (Luminex corporation) as described previously.[25] Search for serum IgG antibodies against HPV16 major capsid protein L1 was performed using ELISA.[27] Serum samples were tested and virus like particle-or E6 bound antibodies were detected using anti-human IgG conjugated to peroxidase (Southern Biotech). For detection of anti-HPV16 E6 antibodies, an *Escherichia coli*-expressed fusion protein composed of HPV16 E6 fused at the C-terminus of glutathione-S-transferase (GST-E6) was generated and coated in microplate wells. For both assays, cut-off value for positivity based on samples from young children (mean reactivity plus 3 SD) was 0.2.

2.7 | In situ immunostaining

Acetone-fixed cryosections were incubated with dextramer (Dextramer-HPV16-PE and Dextramer-HIV-1 PE (Immudex)) for 75 minutes at room temperature and mounted with VECTASHIELD® Mouting Medium with DAPI (Vector Laboratories). Slides were studied using an Axiovert 200 M microscope with MRm camera (Zeiss).

2.8 | Statistical analysis

Data are presented as means ± standard error of the mean. Prism software (Graph Pad) was used for statistical analysis. Statistical analysis was performed by unpaired Mann–Whitney test. A p value of less than 0.05 was considered significant for all analyses.

3 | RESULTS

3.1 | Characteristics of studied patients

To assess whether HPV16 is implicated in other form of LP than its erosive oral form, we investigated patients with LP, excluding patients with erosive oral LP, and patients with LSA, another cutaneous and mucosal dermatosis. The LP patients included had a mean age of 50.8 years (range: 39–55) and a mean duration of LP of 12 years, and presented with lesions of papular LP, bullous LP, hypertrophic LP, non-erosive oral LP and/or lichen planopilaris (Table S1 and Figure S1). Among the 10 studied LP patients, seven had oral lesions and three LP patients had no oral lesions (LP#1, LP#2 and LP#9, Table S1). The LSA patients had a mean age of 58.1 years (range: 28–76) and LSA affected the vulva or the glans, evolving for a mean duration of 7.4 years and treated with topical steroids (Table S2).

3.2 | Circulating $CD8^+$ T-cells of LP and LSA patients display a cytotoxic phenotype

We analysed the phenotype of peripheral blood $CD3^+CD8^+$ T-cells from these patients using flow cytometry, and we monitored granzyme B, perforin and CD107a contents among these cells (Figure 1A). We found that the proportions of circulating $CD3^+CD8^+$ T-cells among total lymphocytes were similar in both LP and LSA patients, and in line with rates observed in healthy donors (HDs) (Figure 1B, top left panel). In contrast, the mean frequencies of $CD3^+CD8^+$ T-cells expressing intracellular granzyme B, perforin or surface

CD107a were higher in LP and LSA patients as compared to HDs (Figure 1B, granzyme B: 24.9 ± 8.1/24.2 ± 4.0/1.1 ± 0.5; perforin: 31.9 ± 9.8/24.3 ± 6.5/4.8 ± 2.2; CD107a: 1.8 ± 0.9/4.5 ± 1.7/0.2 ± 0.1 for LP, LSA and HD, respectively). Of note, no significant differences were observed between LP and LSA patients for all the parameters tested (Figure 1). Overall, we observed that peripheral $CD3^+CD8^+$ T-cells from LP and LSA patients exhibit a cytotoxic phenotype.

3.3 | Peripheral blood $CD8^+$ T-cell repertoire from LP and LSA patients are characterized by $TCRV\beta 3^+$ and $TCRV\beta 6^+$ oligoclonal expansions, respectively

Next, we assessed TCRVβ gene segment usage in sorted $CD4^+$ and $CD8^+$ blood T-cells from the LP and LSA patients and from HD. Interestingly, we detected a predominant usage of the TCRVβ3 gene segment in circulating $CD8^+$ T-cells of LP patients (14.76% ± 3.18%), while this gene segment was used at a much lower level in circulating $CD4^+$ T-cell counterparts from the same patients (6.98% ± 1.02%), and in $CD8^+$ blood T-cells from sex- and age-matched HD (8.47% ± 1.90%) (Figure 2A). Interestingly, the TCRVβ3 usage in $CD8^+$ T cells was similar and not significantly different in the seven LP patients with oral lesions as compared to the three LP patients with no oral lesions (data not shown). Regarding LSA patients, they did not display skewed TCRVβ3 gene segment usage, since it was used at 5.07% ± 0.79%, 5.37% ± 1.37%, and 6.36% ± 1.53% in $CD4^+$ and $CD8^+$ T-cells from LSA patients and in $CD8^+$ T-cells from matched HD, respectively (Figure 2A). In contrast, TCRVβ6 gene segment usage was largely increased in peripheral $CD8^+$ T-cells from LSA patients (15.24% ± 2.9%), while it was detected at similar levels in circulating $CD4^+$ counterparts from the same patients (7.24% ± 0.26%), in $CD8^+$ T-cells from HD (11.30% ± 0.70%) and in $CD4^+$ (7.73% ± 0.18%) and $CD8^+$ T-cells (9.48% ± 0.93%) from LP patients (Figure 2A).

For each TCRVβ gene segment tested from $CD4^+$ and $CD8^+$ T-cells of HD or from $CD4^+$ T-cells isolated of LP and LSA patients, CDR3β length distribution profiles displayed Gaussian-like curves, the hallmark of a polyclonal T-cell repertoire (Figure 2B). In contrast, CDR3β distribution profiles of the TCRVβ3 gene segment were altered with oligoclonal expansions in blood $CD8^+$ T-cells from all LP patients, as shown in Figure 3B top panel, for LP#2 for a CDR3β size of eight amino-acids (AA). Similarly, CDR3β distribution profiles of the TCRVβ6 gene segment were altered with oligoclonal expansions in blood $CD8^+$ T-cells from all LSA patients, as shown for LSA#1 for a CDR3β size of 8 AA (Figure 2B, bottom panel). We next determined the Vβ-Jβ rearrangements corresponding to the observed oligoclonal expansions (Figure 3), and further cloned and sequenced them. As shown in Table 1 and Table 2, one or two dominant CDR3β sequences were detected in each patient. Overall, neither CDR3β length nor consensus sequence was shared across all patients. However, all detected T $CD8^+$ oligoclonal expansions for LP patients were using the TCRVβ3 gene segment, even in patients not sharing common class I HLA alleles (Tables S1 and S2). Notably, 7 out of the 10 patients displayed Vβ3-Jβ2.7 clonotypic TCR and were found irrespective of whether they exhibited oral lesions or not (Table 1). Regarding LSA patients, 4 out of the 10 patients displayed Vβ6-Jβ1.1 clonotypic TCR (Table 2), but neither CDR3β length nor CDR3β AA sequences were found similar in the different patients.

Overall, this set of data shows that LP patients display $CD8^+$ T-cells with a skewed TCR repertoire towards the usage of a restricted set of TCRVβ segments including TCRVβ3, mainly Vβ3-Jβ2.7 oligoclonal expansions. This skewed TCRVβ3 CTL repertoire was not found in patients with another cutaneous and mucosal dermatosis such as LSA.

TABLE 1 CDR3β sequences in PBMC from LP patients.

	CDR3β (nucleotide sequence)[a]	Jβ	Deduced CDR3	CDR3 length	Freq (%)[b]
LP#1	AGTCGGACGGTAAACACCATA	1.3	SRTVNTI	7	29.6
	AGTTTGGTTGAAACAGGTTTTATAGGGACTACGTT TGAGGTTGAGCAG	2.7	SLVETGFIGTTFEVEQ	16	11.1
LP#2	AGTTTGGGTGTGGGCTACGAGCAG	2.7	SLGVGYEQ	8	37.5
LP#3	AGTCTACGGGGGGCCTACGAGCAG	2.7	SLRGAYEQ	8	30.5
LP#4	AGTTTTCAGGGGTTCGAGACCCAG	2.5	SFQGFETQ	8	14.3
LP#5	AGTTTATCGACTCCTAACTACGAGCAG	2.7	SLSTPNYEQ	9	100
LP#6	AGTTTTCAGGGGTACTACGAGCAG	2.7	SFQGYYEQ	8	4
LP#7	AGTTGGACTAGGACTTACAATGAGCAG	2.1	SWTRTYNEQ	9	8
LP#8	AGTTTTGGTCTTCGAGCGGGGACCTACGAGCAG	2.7	SFGLRAGTYEQ	11	54.5
LP#9	AGTTTGACTAGCGCCACCGGGGAGCTG	2.2	SLTSATGEL	9	14.8
LP#10	AGCCTCCGGGGGGCTATTAACGGGGAGCTG	2.2	SLRGAINGEL	10	34.1
	AGTTTATGGGCAGGGAATTTACGTGAGCAG	2.7	SLWAGNLREQ	10	17.1

[a]For each pool, 22–52 bacterial clones were sequenced.

[b]Sequence occurrence/total number of sequences performed, shown as a percentage number.

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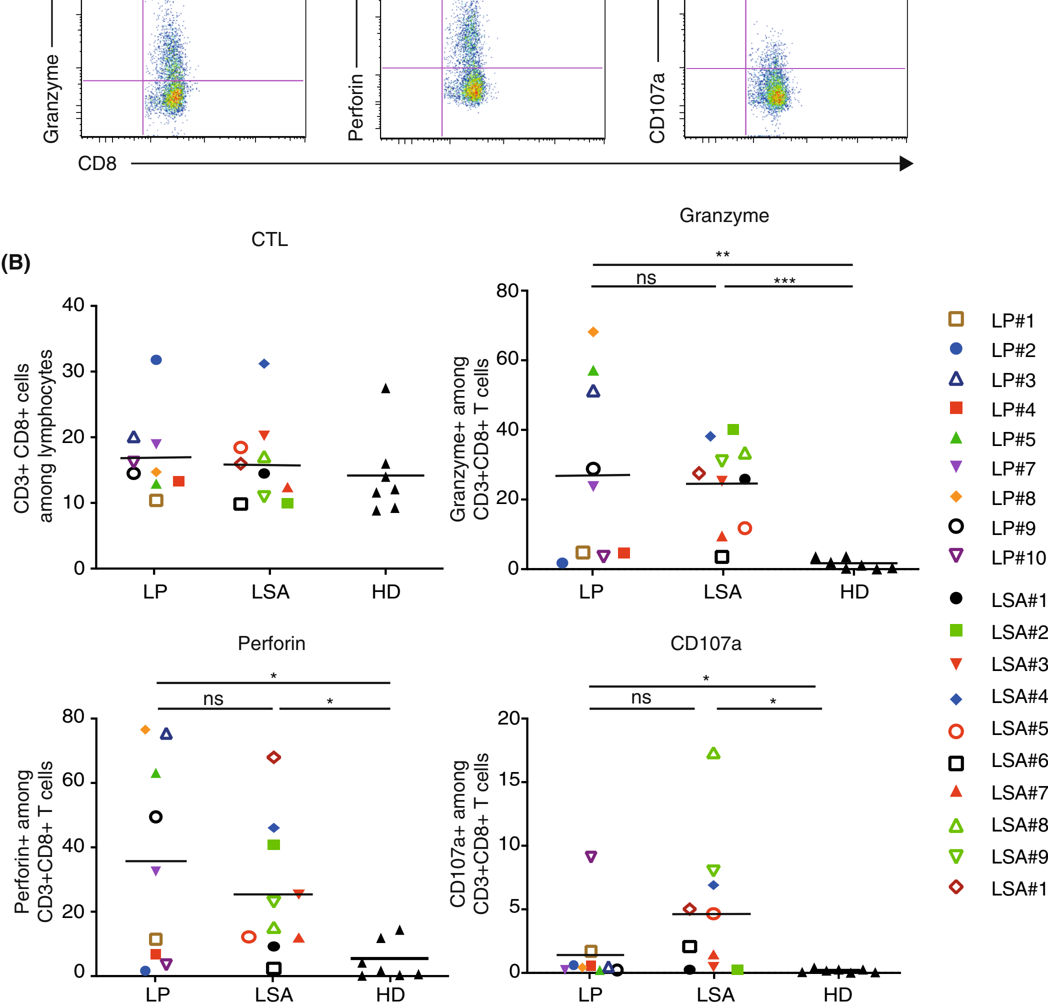

FIGURE 1 Peripheral $CD3^+CD8^+$T-cells in LP and LSA patients express more granzyme B, perforin and CD107a than $CD3^+CD8^+$T-cells from healthy donors. (A) Flow cytometry detection of granzyme B, perforin and CD107a in CTL ($CD3^+CD8^+$ cells) from the blood of a patient with LP. (B) Frequency of CTL in blood and of granzyme B, perforin and CD107a in CTL from the blood of 10 LP, 10 LSA and 7 HD. (*$p<0.05$; **$p<0.01$; ns, non-significant)

3.4 | $CD8^+$ T-cells expressing oligoclonal TCRVβ3^+ in LP patients are specific for HPV16 and found in LP lesions

We assessed current and past HPV16 infection status in the studied patients. Detection of the various HPV genotypes in LP and LSA skin and/or mucosal lesions, sampled at the time of flaring, was performed in all patients, and HPV16 DNA was not found in any patient. Nevertheless, we detected capsid L1-specific IgG in sera of 9 out of 10 LP patients and of 7 out of 10 LSA patients (Figure 4A). Specific IgG against oncoprotein E6 in the sera were detected, always with associated capsid L1-Ig, only in LP patients (3 out of 10 patients, 30%) (Figure 4A). All of these patients had oral mucosal LP lesions and none, an associated HPV-driven cancer.

Further, to test whether TCRVβ3^+ clonal expansions exhibit HPV16-specificity, we stained PBMC from 3 HLA-A*0201$^+$ LP patients of the cohort with peptide-containing MHC class I dextramer. As control and as expected, almost no TCRVβ3^+ T-cells were stained with HLA-A2/HIV-p17 Gag dextramer in these HIV negative patients (Figure 4B). In contrast, a distinct population of $CD8^+$ T-cells expressing a TCRVβ3 specific for the $E7_{11\text{-}20}$ immunodominant peptide of HPV16 was detected in the three tested patients, as shown for LP#10 (Figure 4B), while almost none was detected in the four HLA-A*0201$^+$ LSA patients (Figure 4C), nor in TCRVβ3 negative cells from both LSA and LP patients (Figure 4C).

We then sorted out HPV16-specific $CD8^+$ TCRVβ3^+ cells as well as their $CD8^+$ TCRVβ3^+ Dext-HPV16^{neg} counterparts from LP#10 and LP#5. Spectratyping analysis and CDR3β sequencing were performed, revealing an enrichment of Dext-HPV16$^+$ populations with the SLWAGNLREQ clonotype for LP#10 and the SLSTPNYEQ clonotype for LP#5 (Figure 4D). In addition, in situ immunostaining analysis of LP lesional skin biopsy from LP#1 was performed. We found $CD8^+$

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FIGURE 2 TCRVβ3$^+$ and TCRVβ6$^+$ oligloclonal expansions are skewing the peripheral blood CD8$^+$ T-cell repertoire from respectively LP and LSA patients. (A) TCRVβ3 and TCRVβ6 usages in CD4$^+$ and CD8$^+$ T-cells from the blood of the 10 LP patients, 10 LSA patients and in CD8$^+$ T-cells from 20 sex- and age-matched HD (B) CDR3β length distributions for CD4$^+$ and CD8$^+$ Vβ3-Cβ rearrangements from one representative LP patient (LP#2) and for CD8$^+$ T-cells from one representative HD. The peak of the eighth codon is marked on the abscissa axis. CDR3β length distributions for CD4$^+$ and CD8$^+$ Vβ6-Cβ rearrangements from one representative LSA patient (LSA#1) and for CD8$^+$ T-cells from one representative HD. ($*p<0.05$, $**p<0.01$)

T-cells bearing TCR specific for HLA-A2/HPV16 $E7_{11-20}$ peptide, as revealed by specific Dext-HPV16 staining, whereas staining with the irrelevant Dext-HIV yielded negative results (Figure 4E). Finally, spectratyping analysis on tissue lesions from LP#3 revealed the infiltrate of T-cells bearing Vβ3-Jβ2.7 of 8 AA, as observed in the blood of this patient, as well as the presence of the SLRGAYEQ clonotype (Figure 4F). Thus, oligoclonal CD8$^+$ T-cells observed in the blood of LP patients are specific for HPV16 and are also found in situ in LP lesions.

4 | DISCUSSION

This study demonstrates that LP patients, presenting with a wide variety of clinical manifestations and irrespective of whether they exhibit oral lesions or not, display TCRVß3$^+$ CD8$^+$ T-cells oligoclonal expansions in both peripheral blood and mucosal lesions, which exhibit a cytotoxic phenotype and recognize the HPV16 $E7_{11-20}$ epitope, suggesting a specific hallmark common to many clinical forms of LP. Interestingly, 7 out of the 10 tested LP patients in our study displayed Vβ3-Jβ2.7 clonotypic TCR, a hallmark that we also previously reported in 5 out of 10 LP patients with erosive oral form.[22] Notably, the Vβ3-Jβ2.7 SxxxxYEQ CDR3 consensus sequence of eight AA length was found in 5 out of 20 patients with LP, including the 10 patients studied herein and the 10 patients with erosive oral LP in.[22] In our previous study, the pathogenic relevance of these clonotypic expansions was also supported by their constant presence in all studied LP patients and by their decrease in peripheral blood from patients entering partial or complete LP remission under extracorporeal photopheresis treatment, becoming even undetectable in some patients, and reappearing concomitantly with LP flare when treatment was stopped.[22]

An increased presence of TCRVβ3 positive cells was also previously reported by others in the mucosal T-cell infiltrates of LP patients.[28] Moreover, intra-lesional CTL from LP patients were suspected to recognize an antigen associated with MHC class I molecule on lesional keratinocytes.[29] Interestingly, the high expression of cytotoxic molecules in peripheral CD8$^+$ T cells of LP patients herein suggests a common phenomenon as observed in patients with chronic viral infections.[30] In this line, several studies have shown the presence of several HPV genotypes in mucosal LP lesions, with a strong association with HPV16 genotype.[5,18,20,31,32] Additionally, in vitro studies showed that stimulation of CTL with the HPV16 $E7_{11-20}$ epitope induces the expansion of clones expressing TCRVβ3.[33,34] We also previously showed that peripheral and intra lesional Vβ3 TCR clonotypes of erosive oral LP patients were enriched in HPV16-specific CD8$^+$ T-cells.[22] We thus reasoned that a similar observation could operate in patients with other LP clinical form than the erosive oral one. Even if a current HPV infection was not shown, almost all (90%) of our studied LP patients have been infected by HPV16, as shown by the detection of capsid L1-specific IgG. The detection of oncoprotein E6-specific IgG, which testifies of an active infection with HPV16, was even found in 30% of LP patients, in the absence of associated HPV-driven cancer. Thus, the patients could have a specific HPV16 infection, even if it was clinically inapparent and not properly related to oral lichen planus. Moreover, since less than 1% of healthy controls harboured E6-specific IgG, this high prevalence in LP patients is in favour of a relevant HPV16 infection.[35] A role for HPV infection in LP does not necessarily require constant tissue viral replication over time. Indeed, an initial viral stimulation might be sufficient to trigger the founder immune response, with autoimmune T-cell expansions related to molecular mimicry, unsequestration of masked self-epitopes or both.[36] In line with this hypothesis, molecular mimicry between HPV16 E7 protein and human self has been revealed by computer-based analyses, providing a rational basis for further investigations of LP lesional CTL regarding their immunoreactivity towards HPV16 versus self-candidate antigens.[37,38]

For patients with a dermatosis also affecting the genital mucosa (LSA), we found that their circulating CD8$^+$ T-cells also displayed

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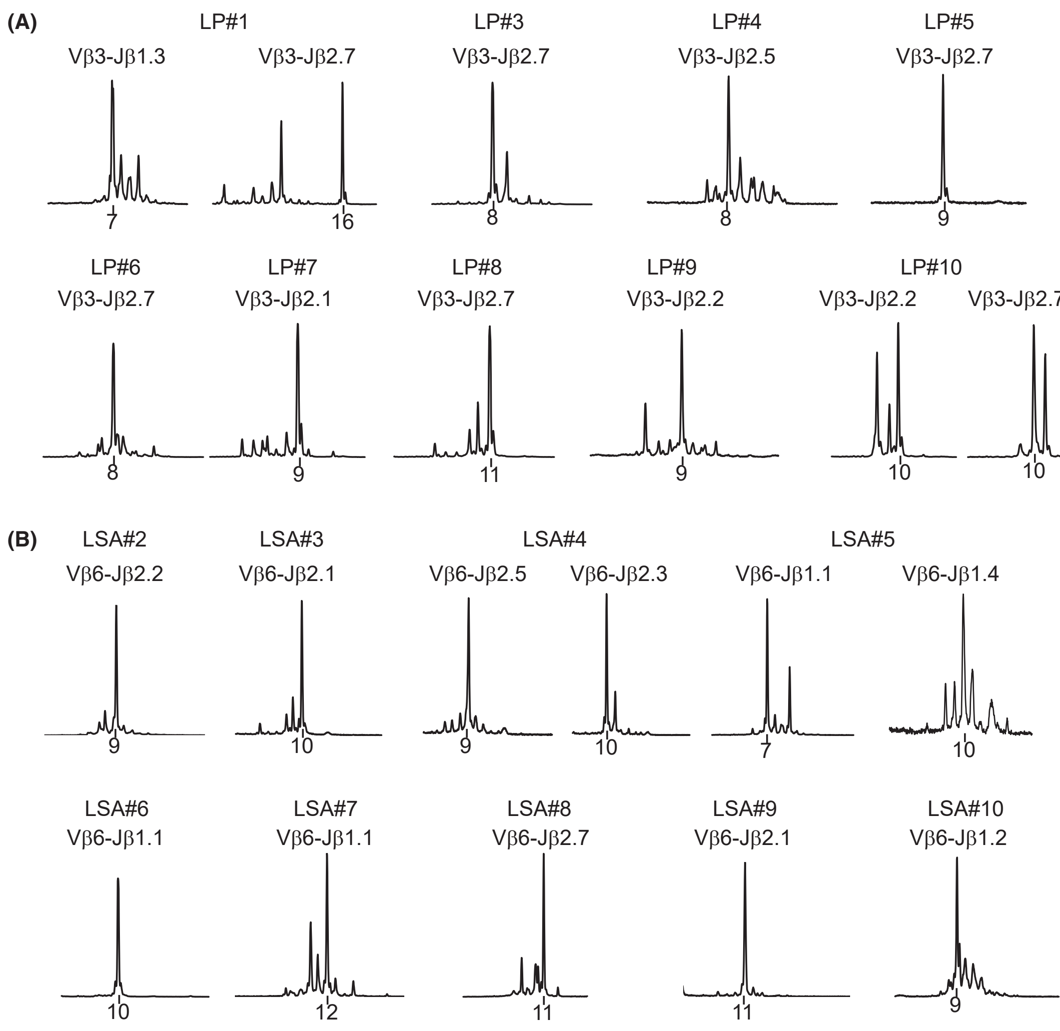

FIGURE 3 Vβ-Jβ Oligoclonal expansions of $CD8^+$ T-cells in the different patients. CDR3β length distributions for CD8+ T-cells from LP (A) and LSA (B) patients. The Vβ-Jβ rearrangement for each patient is indicated. The peak of the codon of the oligoclonal expansion is marked on the abscissa axis.

TABLE 2 Vβ6 CDR3β sequences in PBMC of LSA patients.

	CDR3β (nucleotide sequence)[a]	Jβ	Deduced CDR3	CDR3 length	Freq (%)[b]
LSA#1	AGCTCCATGGGGGTCACTGAAGCT	1.1	SSMGVTEA	8	72.7
LSA#2	AGCTTTTGGCAGGTCCCCGGGGAGCTG	2.2	SFWQVPGEL	9	55.9
LSA#3	AGCCCATACAGGAGCTCCTACAATGAGCAG	2.1	SPYRSSYNEQ	10	66.7
LSA#4	AGCTCGACTAGCTGGGGAGAGACCCAG	2.5	SSTSWGETQ	9	33.4
	AGCTTAGGCCGAGGCCCCGGAGATACGCAG	2.3	SLGRGPGDTQ	10	48.5
LSA#5	AGCCACCAAACGGATGAAGCT	1.1	SHQTDEA	7	17.9
	AGCACTAGGACAGGGCGGTTTGAAAAACTG	1.4	STRTGRFEKL	10	17.9
LSA#6	AGCTCAGCGGGACAGGGCTACACAGAAGCT	1.1	SSAGQGYTEA	10	57
LSA#7	AGCTCACCGACAGGGGGCTTGGATGAAGCT	1.1	SSPTGGLDEAFF	12	33.3
LSA#8	AGCCCCCCCACGGGCGACTACGAGCAG	2.7	SPPTGDYEQYF	11	22.7
LSA#9	AGCTCCAATACGGCAAGCTCCTACAATGAGCAG	2.1	SSNTASSYNEQ	11	50
LSA#10	AGCAATTCCACGTCGGGCTATGGCTAC	1.2	SNSTSGYGY	9	40

[a]For each pool, 18–43 bacterial clones were sequenced.

[b]Sequence occurrence/total number of sequences performed, shown as a percentage number.

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(A)

LP	capsid L1	oncoprotein E6	LSA	capsid L1	oncoprotein E6
#1	+	-	#1	+	-
#2	+	-	#2	+	-
#3	+	+	#3	-	-
#4	+	+	#4	-	-
#5	+	-	#5	+	-
#6	+	-	#6	+	-
#7	-	-	#7	+	-
#8	+	-	#8	+	-
#9	+	-	#9	+	-
#10	+	+	#10	-	-

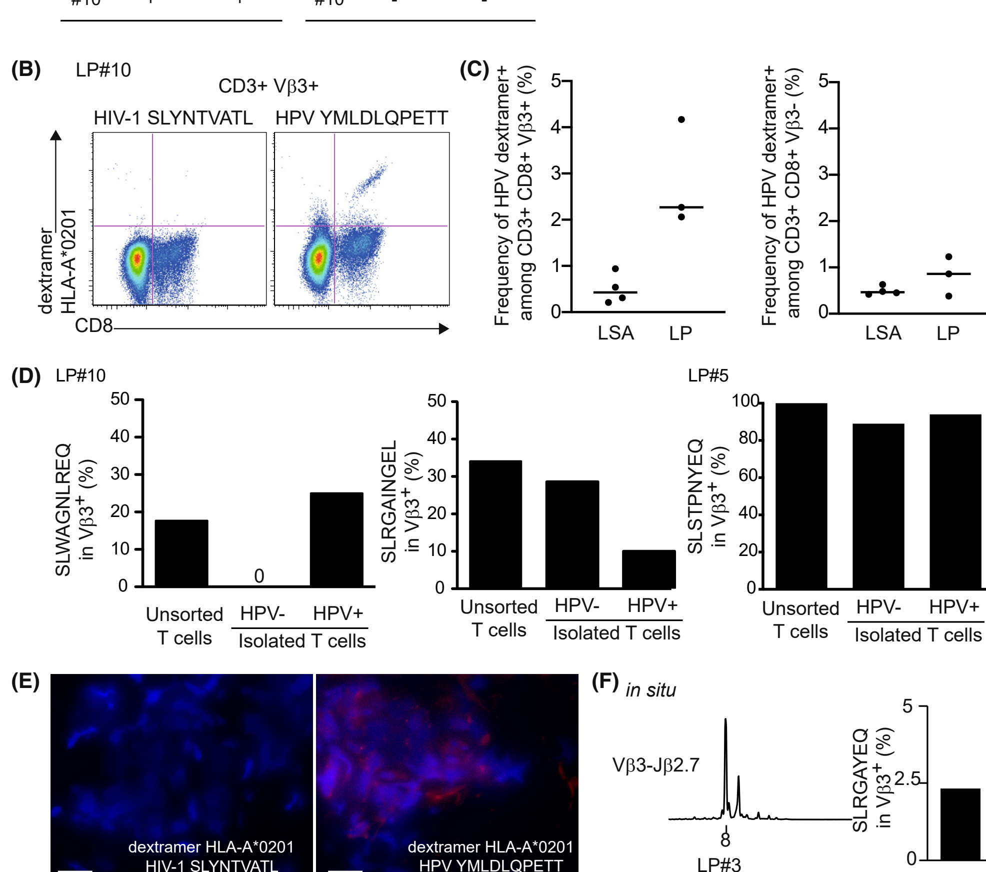

FIGURE 4 A subpopulation of peripheral blood CD8$^+$ T-cells from LP patients is HPV16-specific, enriched with TCRVβ3 clonotypes and detected in lesional skin. (A) Antibodies against capsid L1 and oncoprotein E6 were monitored by ELISA in the sera of the studied patients. (B) Flow cytometry detection using dextramer of HIV- and HPV16-specific CD8$^+$ T-cells in the blood of one representative HLA-A*0201$^+$ LP patient (LP#10). (C) Quantification of HPV-specific Vβ3$^+$ or Vβ3negative CD8+ T-cells in the blood of four HLA-A*0201 LSA patients and three HLA-A*0201 LP patients. (D) Clonotype distribution estimated by CDR3β sequencing for unsorted blood T-cells or isolated blood T-cells with HPV16-dextramer for two different representative HLA-A*0201$^+$ LP patients (LP#10 and LP#5). (E) Skin in situ immunostainings using either dextramer HIV-1 (left) or dextramer HPV16 (right) (x1000 magnification, scale bars = 10 μm) of one representative HLA-A*0201$^+$ LP patient (LP#1) out of the two HLA-A2$^+$ LP patients that were tested. (F) CDR3β length distribution and clonotype distribution estimated by sequencing in skin lesions in LP#3.

a cytotoxic phenotype. Moreover, the peripheral repertoire of CTL was also oligoclonal but not skewed towards TCRVß3+ but TCRVß6+. These observations suggest the involvement of a chronic viral infection, which we found not to be HPV16 but still remains to be identified.

The present findings, as well as those we reported in erosive oral LP,[22] could be limited by the small number of studied patients. After having demonstrated in further and upcoming experiments in a larger number of patients that peripheral TCRVß3+ oligoclonal expansions are strictly specific to LP and that they are not observed in patients with mimicking diseases, such as mucous membrane pemphigoid or other inflammatory dermatoses affecting the scalp or the nails such as lupus or psoriasis, our findings could have several clinical and therapeutical implications for patients affected with inflammatory cutaneous and mucosal diseases. For instance, when clinical and pathological features are not sufficient to confirm LP diagnosis, notably in erosive oral lesions or in some nail and scalp localizations difficult to diagnose, immunological markers such as blood expansions of TCRVß3 CD8$^+$ T-cells or in situ detection of HPV16-specific T-cells could help establishing LP diagnosis and rule out differential diagnosis. Our findings could also pave the way for innovative prophylactic and therapeutic strategies for LP. On an

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epidemiological perspective, it would be interesting to test the influence of the introduction of HPV16 vaccination, in particular in countries with high vaccination coverage such as Australia, on the incidence of LP. In terms of therapeutics, we can assume that the use of antiviral treatments with an HPV-specific effect, such as cidofovir,[39] would have a limited impact on the course of LP since complete viral clearance is not expected, and a late antiviral intervention is unlikely to reverse the autoimmune process. However, deletion or anergization of pathogenic T-cells through immunotargeting of the $CD8^{+}TCRVß3^{+}$ subset, could have a therapeutic impact in LP, and the development of anti-TCRVß3 monoclonal antibodies as well as of bispecific CD8/Vß3 immunoreactants could be of interest for the treatment of refractory erosive oral LP that frequently requires long-term association of immunosuppressive drugs.

Overall, while patients with LP exhibit a wide variety of clinical manifestations, clonal peripheral and intra-lesional HPV16-specific $CD8^{+}$ T-cells pinpoint a specific hallmark common to different clinical forms of LP that could be used in clinical settings in the future.

AUTHOR CONTRIBUTIONS

MV, MLG and NF conceived and designed the studies. CP, BP, MB, FA, HB, JLP, TG, MT, AT included patients or carried out experiments. MV, FA, HB, JLP, AT, MLG and NF wrote the manuscript.

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CONFLICT OF INTEREST STATEMENT

All authors declare no competing interests.

DISCLAIMER

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Figure S1

Tables S1–S2

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