Bull Group Int Rech Sci Stomatol Odontol 40, 92-97, 1998

Presence of HPV 16 sequences in oral lichen planus lesions

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Key words: HPV, oral Lichen planus, PCR
Mots clés: HPV, Lichen plan buccal, PCR

RESUME

Le lichen plan buccal est une altération de la muqueuse buccale d'orgine inconnue. L'infection de HPV a été suggérée comme un possible facteur étiologique. Nous avons étudié 17 lésions de lichen plan buccal en utilisant la PCR — une puissante technique pour la détection des séquences d'ADN de différentes lésions — pour détecter HPV–16–ADN séquences. Nous avons trouvé un pourcentage de 11. 76% de lésions de lichen plan buccal HPV–16 positives (2 de 17). Dans les études histopathologiques nous n'avons trouvé aucun effet cytologique d'infection de HPV. Il est possible que l'état intégré de HPV–16–ADN soit responsable de la perte de la capacité de réplication, et par conséquent des effets biologiques de la réplication virale.

INTRODUCTION

Oral lichen planus (OLP) is a chronic inflammatory mucosal disorder of unknown origin (Scully and El-Korm, 1985). Nevertheless, diverse exogenous agents including generalized medical disorders, mainly diabetes, hypertension and liver disorders (Tyldesley, 1974; Lacy et al., 1983); systemic medications (Robertson and Wray, 1992); viral infections (Maitland et al., 1987; Biersterfeld et al., 1991) and a number of immunologic disorders (Walsh et al., 1990) are often associated with the initial onset and subsequent exacerbations of OLP lesions.

The possible viral etiology is also proposed by the recent demonstrations of the presence of various viruses like HSV-1 and HPV in a high percentage of oral lesions including OLP (Tyldesley, 1974; Maitland et al., 1987; Cox et al., 1993). Despite these studies on the presence of HPV in OLP lesions, the real etiological role of this virus remains to be established. HPV infections have been demonstrated in several oral disorders (Löning et al., 1985; Syrjänen et al., 1986; Milde and Löning, 1986; Eversole and Lairpis, 1988; Scully et al., 1988; Syrjänen et al., 1988; Greer and Shroyer, 1990; Zeuss et al., 1991; Young and Min, 1991); however, detection rates varied, dependent on the type of study and especially on the method involved.

HPV types 16 and 18 have a well-established association with malignant progression and invasive carcinoma of the uterine cervix (Boshart, M. et al., 1984; Gissmann, L. et al., 1984; Lörincz, A.T. et al., 1986, 1987; Gupta, J. et al., 1987). Several reports have shown that oral dysplastic epithelium and oral squamous cell carcinomas contain high-risk HPV DNA types 16 and 18 (Löning, T. et al., 1985; De Villiers, E.M. et al., 1985; Milde, K. and Löning, T., 1986; Dekmezian, R.H. et al., 1987; Syrjänen, S.M. et al. , 1988; Kashima, H.K. et al., 1990; Greer, R. and Shroyer, K., 1990; Hönig, J.F., 1992; Hönig, J.F. et al. , 1995). These high-risk HPV types were seen in oral precancerous lesions (De Villiers, E.M. et al., 1985; Adler-Storthz, K. et al., 1986; Scully, C. et al. , 1988; Syrjänen, S.M. et al. , 1988; De Villiers,

E.M., 1989; Brandsma, J.L. and Abramson, A.L., 1989; Greer, R. and Shroyer, K., 1990; Watts, S.L. et al., 1991). So far, HPV-6, -11 and HPV 16-related virus have been found in OLP lesions (Syriänen, S.M. et al., 1986; Maitland, N.J. et al., 1987). Our previous studies have shown the presence of HPV DNA types 6/11, 16/18 and 31/33/35 in different lesions of the oral mucosa using the in situ hybridization technique (ISH) (Gonzalez-Moles, N.A. et al., 1994).

The present study was undertaken to examine the presence of HPV type 16 in OLP lesions using the polymerase chain reaction (PCR), a more sensitive and specific molecular technique than ISH.

MATERIAL AND METHODS

Patients and protocols

Formalin-fixed paraffin-embedded oral biopsies from 17 patients with OLP lesions attended in the Oral Medicine Department of School of Dentistry of Granada were included in this study. 11 patients were women (mean age = 45.36 yr, range = 20-72 yr) and 6 were men (mean age = 45.16 yr, range = 21-72).

Clinical parameters collected include lesion type, symptoms, lesion location and other related factors. The histopathological examination included observation of epithelial alterations, basal layer vacuolization, intensity of the inflammatory infiltrate, number of Civatte's bodies and intraepithelial lymphocytes, and the presence de Max-Joseph's spaces and koilocytic cells. Histopathological study was performed by the same oral pathologist and quantifying of basal layer vacuolization and intensity of inflammatory infiltrate was based on his personal experience.

Cell culture

The CaSki cell line was obtained from the American-type culture collection (Rockville, MD) and was cultured in an RPMI 1640 medium supplemented with 10% faetal calf serum. CaSki contains HPV 16 sequences (Howley, P.M., 1987).

Amplification of HPV sequences

Multiple (3 to 5) 10 µm sections of the paraffin-embedded tissues obtained from each patient were packed into 0.5 ml. Eppendorf microcentrifuge tubes, dewaxed with xylene, washed with ethanol and digested for 24 h at 37°C in an extraction buffer containing 50 mM KCl, 10 mM Tris- HCl (pH:8.3), 2.5 mM KCl, 0.1 mg/ml gelatin, 0.45% Nonidet-P40, 0.45% Tween 20 and 60 µg/ml proteinase K. The samples were heated to 95°C for 8 min to inactivate the proteinase K, centrifuged (10,000 g x 10 min) to pellet the debris, and 10 µl of the supernatants were analyzed by PCR. PCR amplifications were performed using a DNA thermal cycler (Perkin-Elmer-Cetus, Norwalk, CT). The total reaction volume was 100 µl, containing 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 1.5 mM $MgCl_2$, 200 µM each of dATP, dCTP, dGTP, and dTTP, together with 1 µM of each primer. Primers specific for a segment of the E6 gene (HPV 16) were synthesized by Eurogentec (Liège, Belgium) (see Tab. 1). Finally, 2.5 units of thermostable Taq polymerase (Amplitaq, Perkin-Elmer-Cetus) were added and the whole mixture was submitted to 32 cycles of amplification with the following profile; denaturation for 2 min at 94°C; primer annealing for 2 min at 55°C, and primer extension for 1 min at 72°C.

PCR assay was performed using both positive and negative controls. Positive controls consisted of DNA extracted from HPVcontaining CaSki cell line.

The reaction products were analysed by a dot-blot hybridization assay using ^{32}P-labelled oligomer probes specific for HPV 16 (Tab. 1) (Seedorf, K. et al., 1985). The filter was pre-hybridized for 1 h at 42°C in 3 x SSPE, 5 x Denhardt's, 0.5% SDS before adding ^{32}P-end-labelled oligomer (Roychoudhury. R. and Wu, R., 1980) and then hybridized for 1 h at 42°C in the same solution. The filter was washed in 1 x SSPE, 0.1% SDS 3 times for 5 min at room temperature, once at 55°C for 10 min and finally again at room temperature for 5 min. Autoradiography

Tab. 1: Oligonucleotide primer and probe sequences used to detect HPV 16 DNA by PCR

HPV type	Primer location	Sequence	Product length (bp)
16-upstream	E6 (320-339)	5'-ATTAGT GAG TAT AGA CAT TA-3'	
16-downstream	E6 (410-429)	5'GGCTTT TGA CAG TTA ATA CA-3'	109
16-probe	E6 (354-393)	5'-ATGGAA CAA CAT TAG AAC AGC AAT ACA ACA AAC CGT GCT G-3'	

Tab. 2: HPV-16 analysis in OLP lesions.

Sample n°	Sex	Age	Lesion type	Symptoms	Site[a]	Related factor	PCR assay HPV-16
1	F	26	atrophic	discomfort	1,3	stress	—
2	F	23	reticular	none	1,3	none	—
3	M	42	reticular	discomfort	1,3,5	stress	—
4	F	70	erosive	pain	1,3,5	none	—
5	F	52	reticular	none	1	none	—
6	F	20	reticular	none	1,3,5	stress	—
7	F	27	atrophic	discomfort	1,2,3,5	none	—
8	M	39	reticular	discomfort	1	stress	—
9	M	72	erosive	pain	1,2,3,5	stress	—
10	F	72	atrophic	pain	1,3	none	—
11	F	70	erosive	pain	1,3	none	+
12	F	51	reticular	none	1	none	—
13	F	59	erosive	pain	1,2,3,4	none	+
14	M	70	reticular	pain	1,2,3,5	stress	—
15	M	27	reticular	none	1	stress	—
16	M	21	reticular	none	1	none	—
17	F	29	reticular	none	1	none	—

[a] Sites: 1. Buccal mucosa, 2. Tongue, 3. Gingiva, 4. Lips, 5. Palate

was performed at -70°C with Kodak X-AR film, and exposed for 1 h. Samples showing amplification below or equal to the negative controls cells were considered negative.

RESULTS

Tab. 2 summarizes the clinical description and the presence of HPV DNA type 16 in the 17 OLP lesions studied using of the PCR technique. Overall, 11.76% of OLP lesions examined (2 out of 17) were found to contain HPV-16-related sequences. The autoradiograph of the PCR amplification product is presented in figure 1.
Positive HPV-16 biopsy specimens came from women and were erosive clinical type of OLP.
Tab. 3 shows the histopathological parameters of OLP lesions analyzed. Positive HPV-16 cases showed a maximal vacuolization of the basal layer and an intense inflammatory infiltrate. No morphologic cellular alterations of HPV infection such as koilocytosis, were found in the positive HPV-16 cases.

DISCUSSION

Oral lichen planus (OLP) is a chronic inflammatory mucocutaneous disease of unknown origin (Scully and El-Korm, 1985). Diverse etiological factors have been

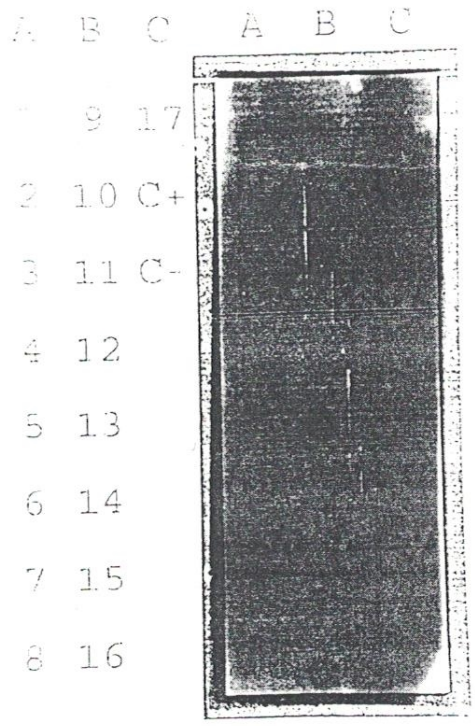

Fig. 1: Detection of HPV sequences in the 17 oral lichen planus lesions. OLP specimens B11 and B13 were coded as positive. C+ and C- refer, respectively, to positive and negative controls

Tab. 3: Histopathologic characteristics of OLP lesions.

Sample n°	Epithelium[a]	Basal layer[b]	Inflammatory infiltrate[c]	Civatte's bodies[d]	Exocytosis[e]	Max-Joseph's spaces	Koilocytosis
1	1	+ + +	+ + +	2-4	11-25	none	none
2	2,3,4,5	+	+	0-1	0-10	none	none
3	1,2,3,4,6	+ +	+ + +	0-1	1-25	yes	none
4	1,7	+ + +	+ + +	2-4	11-25	yes	none
5	2,3,4,5	+	+ +	0-1	0-10	none	none
6	2,3,4,6	+ +	+ + +	2-4	11-25	none	none
7	1,2,3	+ +	+ +	2-4	>25	yes	none
8	2,3,4,5	+	+ +	0-1	0-10	none	none
9	1,4,6,7	+ + +	+ + +	2-4	>25	yes	none
10	1,7	+ + +	+ + +	2-4	11-25	yes	none
11	1,7	+ + +	+ + +	2-4	11-25	yes	none
12	2,3,4,5	+	+ +	0-1	11-25	none	none
13	1,7	+ + +	+ + +	0-1	11-25	yes	none
14	1,2,3,4,6	+ +	+ + +	0-1	0-10	yes	none
15	2,3,4,6	+ +	+ +	0-1	11-25	yes	none
16	2,3,4,5	+ +	+ +	0-1	0-10	none	none
17	2,3,4,5	+ +	+ +		0-10	none	none

[a] Epithelium: 1. atrophic, 2. hyperplasic, 3. acanthosis, 4. hyperkeratosis, 5. parakeratosis, 6. orthokeratosis, 7. erosive.
[b] Basal layer: + minimal, + + moderate, + + + intense vacuolization. [c] Inflammatory infiltrate: + mild, + + moderate, + + + intense.
[d] Civatte's bodies: n° of Civatte's bodies/8 fields/40x. [e] Exocytosis: n° of intraepithelial lymphocytes/8 fields/40x.

related with this disorder and HPV infection has recently been suggested (Jontel, M. et al., 1990).
The present authors found 11.76% of OLP lesions to contain HPV-16-related sequences. This rate is included in recently published data. Cox et al., 1993 found 75% of cases to be positive for HPV-16 (3 out of 4) using in situ hybridization; Syrjänen et al., 1986 found one positive case with the same technique. Other researchers found no HPV positive OLP cases (Kashima, H.K. et al., 1990).
Jontell et al., 1990 using PCR, founded 65% of erosive OLP cases to contain HPV DNA types 6,11, 16 or 18 and 15% erosive OLP containing HPV-16-related sequences. In the present authors' series, all of the OLP lesions which tested HPV- 16 positive were of the erosive type.
It is generally believed that basal cells are the targets for HPV infection. The viruses may get direct access to the basal cells if exposed to a wound or an abrasion (Chang F. et al., 1991). This fact might explain that only erosive forms of OLP lesions were found positive for HPV-16 in the present study. However, erosive OLP is usually resistant to permanent cure and requires regular steroid treatment and examinations since this form is considered to be a premalignant condition (Barnard N.A. et al., 1993).
Steroids are known to decrease the density of Langerhans cells which express class II antigens thereby opening ways to viral antigens (Jontell M. et al., 1988).
In addition, a transcriptional activation element inducible by glucocorticoids has been identified in the regulatory regions of the HPV-16 genome which, in treated patients with virus could lead to over-expression of HPV transforming genes (Pater M.M. et al., 1988).
Moreover, histophatological examination of HPV-16 positive specimens showed no morphological characteristics of HPV infection such as koilocytosis (Greer R. et al., 1987). Koilocytosis is suggested to be a cytopathic effect of HPV and shows productive HPV infection (Koss, L.G. and Durfee, G.R., 1956). HPV-16 DNA is usually integrated into the cellular genome loosing its replicative capability. This might, perhaps be a possible explanation for the absence of koilocytosis in HPV-16 positive OLP lesions (Jontell, M. et al., 1990).
Thus, further evidence is required in order to uncover an etiological role for HPV-16 in the pathogenesis of OLP.

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