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Oral Hairy Leukoplakia: Pathogenetic Aspects and Significance of the Lesion

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Oral hairy leukoplakia in HIV-seropositive persons is considered as a highly serious sign and places the patient in the AIDS-related complex group according to the classification recommended by the Centers for Disease Control. Epstein-Barr virus (EBV) is thought to be the cause. Based on the investigation of 14 of our own cases and a review of the literature, we conclude that so called hairy leukoplakia does not have a specific histopathologic pattern. Identical lesions can be caused by fungus infection, or biting and other kinds of mechanical irritation. Both fungal infection and EBV infection have been proven in a high percentage of the lesions. However, EBV has been found also in apparently normal oral mucosa. This questions the assumption that the virus is the cause of the lesions. In our investigation the presence of "hairy leukoplakia" did not reflect the clinical status of the patient. The best indicator of the clinical status was the T-lymphocyte subset CD4+ number in the peripheral blood. It appears that low CD4+ counts, candidiasis and the presence of replicating EBV in the epithelial cells are parallel markers of increasing immunodeficiency. ***Key words: HIV; EBV; Candidiasis; Histopathology; Oral epithelium.***

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In 1984, Greenspan et al. coined the concept of hairy leukoplakia (HL) (1) in a study of 37 homosexual men with corrugated or hairy lesions located on the lateral borders of the tongue. The authors thought this kind of lesion to be a new entity, which occurred exclusively in male homosexuals. They found the histopathologic pattern of these lesions consistent, distinctive and different from that of any previously defined oral white lesion and particularly emphasized the presence of hairy projections from the surface and ballooned and vacuolated cells in the epithelium. The authors also found evidence for the presence in prickle cells of human papilloma virus and of a herpes group virus, later on identified as Epstein-Barr virus (EBV) (2). They also concluded that the EBV was able to replicate within the epithelial cells. *Candida* was found in a high percentage, but HL was thought to be an entity separate from candidiasis. In a third paper in 1987 Greenspan et al. (3) discussed the relation of HL to infection with human immunodeficiency virus (HIV) and the risk of developing acquired immunodeficiency syndrome (AIDS). Survival analysis showed that the probability of AIDS developing in patients with HL was very high. The authors concluded that HL was highly predictive of the development of AIDS. In consequence, patients with HL were, according to the classification recommended by the Centers for Disease Control (CDC), included in the group IVC-2 or AIDS-related complex (ARC), the group most closely related to full-blown AIDS, and therefore HL was to be considered to indicate a poor prognosis (4).

After the first reports of Greenspan and co-workers there have been numerous investigations on HL on record, and most of the statements made by Greenspan and co-workers have been disproved or called in question both by themselves and by others. We have found the histopathologic pattern described as unique for HL very similar to that observed in and thought to be pathognomonic for biting lesions, *morsicatio mucosae oris* (5). Moreover the presence of ballooned cells in the epithelium is considered to be a normal phenomenon in the buccal mucosa (6). According to Cawson & Eveson (6), the dorsal surface of the tongue has a thick epithelium characterized by closely set filiform papillae interspersed with scattered fungiform papillae. The filiform papillae produce 3–4 keratinized chords, each of them ending up in cornified tips, called hairs. In contrast the ventral aspect has a thin, even, and non-keratinizing epithelium. However, even though some textbook illustrations clearly show that the epithelium mostly is light, we have found no comments on this or on the appearance of vacuolated and ballooned cells. The feature of the normal tongue border has been studied by Andersen et al. in 1990 with special reference to HL (7). Macroscopically they found that the border displayed parallel, vertical mucosal ridges alternating with shallow grooves from the foliate papillae to the apex of the tongue. Though these mucosal folds could contain an occasional papilla there was a distinct boundary line between the dorsal aspect and the border area of the tongue. Microscopically they found that sections cut perpendicular to mucosal folds showed a regular surface with domeshaped protrusions alternating with shallow grooves, whereas sections cut parallel to the ridges disclosed an irregular configuration due to tangentially cut folds, and thus resembled epithelium described as typical of HL.

The aim of our investigation was to: 1) review the literature on HL; 2) compare the histopathologic pattern of unremovable white lesions clinically suspected to be HL in HIV-seropositive patients with that of biting lesions in healthy persons, and with the histologic appearance of the normal tongue mucosa.

REVIEW OF THE LITERATURE

Affected individuals

Lesions said to be clinically and/or histopathologically typical of HL have been observed in other groups of HIV-seropositive individuals in addition to homosexual men (8), in HIV-seronegative, but for other reasons immunodeficient patients (9, 10),

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and also in HIV-seronegative healthy persons, not at risk of HIV infection (11, 12).

Clinical appearance and location

HL was first described as unremovable corrugated or hairy lesions located on the lateral borders of the tongue. Later, lesions have been described also on the ventral and dorsal aspects of the tongue, on the buccal mucosa and on the palate (13). Lesions on the ventral aspect of the tongue were often flat. Generally they were asymptomatic. The size of the lesions varied considerably. There was no correlation between the size or the severity of HL and the development of AIDS (14).

Microscopic appearance

Greenspan and co-workers described the epithelium as acanthotic and slightly parakeratotic, provided with slender keratotic projections resembling hairs. They also found what they called characteristic ballooning changes in the squamous cell layer. These cells were vacuolated with clear cytoplasm and pycnotic nuclei surrounded by a halo. They were predominantly situated in the superficial part of the *stratum spinosum* but in a few cases above the basal cell layer. The authors compared the changes with dermal warts and cervical condylomata and called the ballooned cells koilocytes. Another observation was the absence of, or very few, inflammatory cells both in the epithelium and in the subepithelial connective tissue in spite of fungal infection (1).

In subsequent investigations on HL the histopathologic pattern has been described as more variable according to the degree of acanthosis and parakeratosis and to the occurrence of "hairs". Many lesions did not have "hairs". Also bacteria covering the epithelial surface without any inflammatory reaction have been added to the histologic pattern (13–15). Ballooned and vacuolated epithelial cells, so called koilocytes, have been found in a high percentage in presumed HL lesions, and in spite of a few critical voices (9, 13), the presence of them has been stressed as essential, or highly suggestive for the establishment of the diagnosis (16–19). In 1986, Eversole et al. (13) compared biopsy material from HL lesions in homosexual men with material from oral candidiasis and oral leukoplakia lesions without cellular atypia in heterosexual individuals. The homosexual group consisted of 36 patients and the two other groups of 25 persons each. They found "koilocytes" in over 85% of the specimens in all three groups and consequently called in question the unique character of ballooned cells in HL. In 1989 Syrjänen et al. (9) described an EBV-positive HL-like lesion on the border of the tongue in an HIV-seronegative but immunocompromised patient. In the biopsy material they found "characteristic ballooning cells", but even so they commented that this kind of cell could be seen also in biopsy specimens from normal tongues. Furthermore they strongly objected to calling the ballooned cells koilocytes, a distinction, they thought, should be made only for cells infected with human papilloma virus.

Candidiasis

In clinically typical HL lesions candidiasis has been observed in 51–100% of the cases (1, 13, 15, 20, 21). In order to separate HL from candidiasis it has been proposed that the diagnosis of HL should not be made if a lesion disappears completely after "adequate antifungal" treatment (14). However, also after this kind of distinction, the percentage of *Candida* hyphae in biopsies from lesions accepted as HL has been found high (14, 16). In the last few years candidiasis in HL lesions has more or less been accepted as a secondary phenomenon, and the important task in establishing the diagnosis HL has become not to exclude candidiasis, but to prove the presence of EBV in the epithelial cells of the lesions (15, 22, 23).

Presence of virus

Further investigations by electron microscopy have not confirmed the presence of human papilloma virus (15, 17, 18, 20, 21). However, there is a wide agreement, based on investigations by immunohistochemistry, electron microscopy and/or in situ hybridization techniques, that the epithelial cells in the upper part of *stratum spinosum* in lesions of HL often are infected with EBV (66–100%), and that the virus is able to reproduce (15–17, 19–21). Furthermore, some investigators are of the opinion that different kinds of intranuclear herpes-type inclusion bodies, mostly located in the koilocytoid cells in the upper part of the squamous cell layer, are the best light microscopic hallmark of HL (15, 17). Therefore EBV has been considered as the cause of HL. That is the reason why today most investigators require the presence of EBV to be proven before the definite diagnosis of HL is accepted (12, 16, 17, 19, 21, 23).

HL as a prognostic marker of AIDS

In 1987 Greenspan et al. (3) stated that the probability of AIDS developing in patients with HL was 48% by 16 months and 83% by 31 months. Contradictory to this statement, in the same year, Schiødt together with Greenspan, Daniels and Greenspan (14) found HL to be a frequent and early clinical sign of exposure to HIV.

In 1991 Greenspan et al. (22) presented the results of an investigation based on a follow-up of 198 patients with HL. They found that the time interval between the appearance of HL and AIDS varied considerably. In 27 patients AIDS had not developed 1000 days after the onset of HL. This group, the slow progression group, was compared with the 28 patients presenting the shortest interval, the fast progression group. In the latter group by definition, all had developed AIDS within 1000 days. The median time to AIDS was 6.4 months. In the slow progression group only 4 patients developed AIDS during an observation time of nearly 6 years. In spite of these contradictory results the authors considered HL as a predictive marker for the development of AIDS.

MATERIAL AND METHODS

Our material consisted of 14 homosexual HIV-seropositive patients who had unremovable white lesions on the lateral border of the tongue clinically suspected to be HL, and who consented to biopsy. Eight patients were outpatients at the Dental Clinic for Infectious Diseases, Huddinge University Hospital, coming for routine follow-up, and 5 were sent to the dental clinic by their physician because of the tongue lesions. In one patient the biopsy specimen was taken at the Dermato-Venereological Clinic, Södersjukhuset, Stockholm. The patients were seen between October 1990 and January 1993.

The histopathologic findings in these cases were compared with the

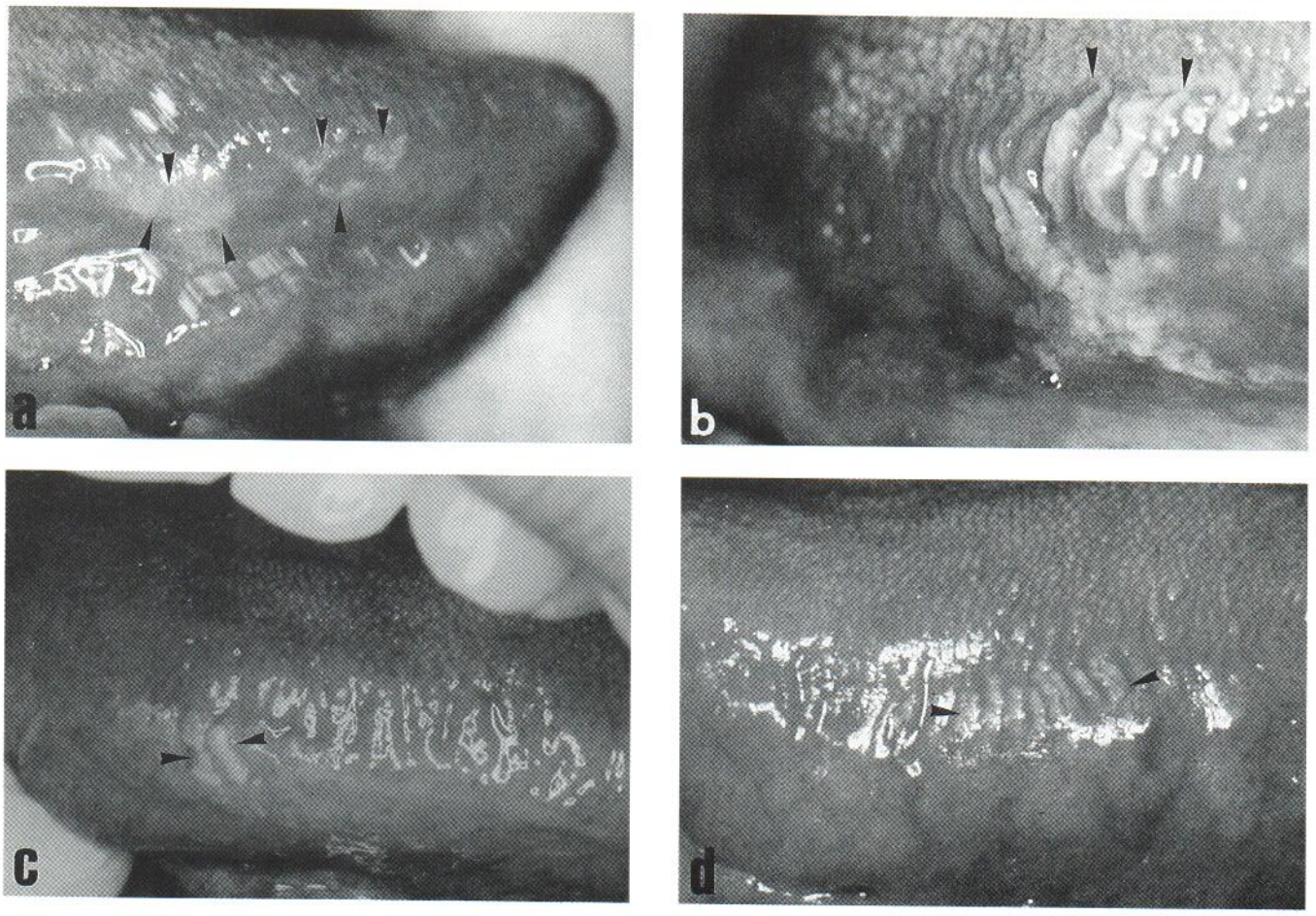


Fig. 1a–d. Tongue lesions in patients D, L, E and K. *Arrow-heads* indicate the lesions on the tongue borders. (*a*) D had multiple flat lesions. (*b*) L had a large and markedly corrugated lesion, which continued on the ventral aspect. (*c*) E had a corrugated lesion on the tongue border opposite a sharp tooth. (*d*) K had a corrugated lesion.

findings in material taken from 3 macroscopically normal tongues at autopsy, and with those in biopsy specimens taken from 5 biting lesions (4 from the buccal mucosa, 1 from the lateral border of the tongue) in 5 healthy persons, not at risk of HIV infection. One biopsy specimen from each patient was obtained either with a punch or a scalpel. The autopsy material consisted of large pieces taken from the anterior part of the tongue and cut at right angles to the border, transversely or longitudinally. All specimens were fixed in 10% buffered formalin, paraffin-

Table I. *The 14 HIV-seropositive men with suspected HL on the lateral border of the tongue, sorted according to CDC-group*

Pat.	Age	CDC-group	CD4+[a]	CD8+[a]	EBV	Fungus	Treatment[b]
A	42	II no sympt	1.57	0.84	neg	neg	5
B	28	II no sympt	0.64	1.97	neg	*Candida*	2
C	22	III PGL	0.44	0.68	pos	*Candida*	8
D	40	III PGL	0.41	1.85	neg	neg	1
E	27	III PGL	0.35	0.92	neg	neg	1
F	30	IV C-2 ARC	0.28	1.14	pos	*Candida*	1
G	38	IV C-2 ARC	0.16	0.59	neg	*Candida*	1
H[c]	31	IV C-2 ARC	0.008	2.24	pos	neg	3
J[c]	47	IV C-1 AIDS	0.14	0.28	neg	neg	4
K[c]	40	IV C-1 AIDS	0.031	0.03	pos	neg	2
L[c]	43	IV C-1 AIDS	0.03	0.45	neg	*Candida*	1
M[c]	49	IV C-1 AIDS	<0.01	0.29	neg	*Candida*	5+6+7
N[c]	29	IV D AIDS	0.01	0.00	pos	neg	1
O[c]	30	IV D AIDS	0.005	0.1	pos	*Aspergillus*	2+4+5

[a] $\times 10^9$/L [normal values: CD4+ cells, 0.34–1.61 $\times 10^9$/L; CD8+ cells, 0.21–1.06 $\times 10^9$/L] (26).

[b] 1 = no drugs at all, or no antiviral or antifungal drugs; 2 = zidovudin, anti-retrovirus; 3 = dideoxyinosine anti-retrovirus; 4 = acyclovir, anti-herpesvirus; 5 = fluconazol, anti-fungus; 6 = itraconazole, anti-fungus; 7 = flucytosin, anti-fungus; 8 = r gp 160 vaccine (Vax Syn HIV-1; Micro Gene Sys).

[c] Deceased. Patient J committed suicide.

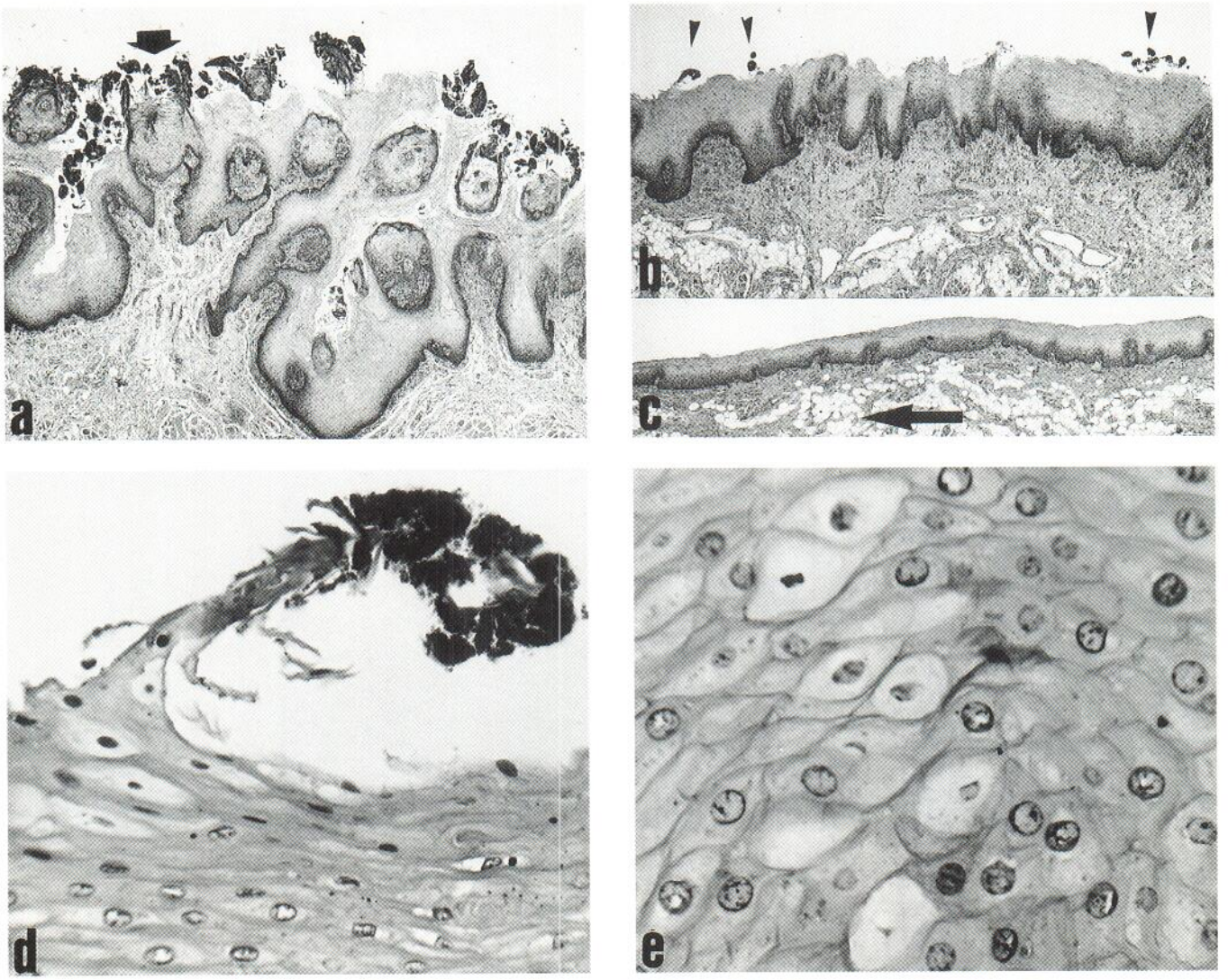


Fig. 2a–e. Normal tongue, a section cut transversely at right angles to the border. (*a*) The dorsal aspect has a light and very thick and irregular epithelium with closely set filiform papillae cut in various directions. The *arrow* indicates a filiform papilla with bacteria-coated cornified "hairs". (*b*) The uppermost part of the border contains a single rudimentary filiform papilla with a "hair", and several cut-off tips of "hairs" (*arrow-heads*). (*c*) The smooth and thin epithelium of the middle part of the border. The *long arrow* in (*c*) indicates the direction towards the ventral aspect. (*d*) The papilla "hair" indicated in (*b*). (*e*) A group of ballooned and vacuolated cells.

embedded and stained with hematoxylin-eosin, PAS, and according to van Gieson. After deparaffinization sections for in situ hybridization were treated with proteinase K (Boehringer, Mannheim, Germany) (25 μg/ml in Tris-buffer) for 20 min and hybridized with an oligoprobe (23-mer) selected from the *Not I* EBV-region and 3'-End labelled with ^{35}S (24). Hybridization was performed according to a protocol described previously (25). Cultured cells were used as positive (B95:8) and negative (Bjab) controls.

T-lymphocyte subsets in the peripheral blood were determined at the Swedish National Bacteriological Laboratory by direct immunofluorescence (26).

RESULTS

Clinical investigations

One or several white unremovable plaques were observed on one (3 cases) or both (11 cases) tongue borders. The lesions were corrugated in 12 patients and flat in 2; the smallest lesion was about 8 mm^2, while the largest covered the whole border. In patient L the lesion continued on the ventral aspect (Fig. 1a–d). In addition to white plaques on the tongue, patient A had bilateral white patches on the buccal mucosa, patients G and B had angular cheilitis, patients L and M had erythematous candidiasis on both the buccal mucosa and the soft palate, patient D had occasional major aphthae, patient N had oral Kaposi's sarcoma, and patient E had a broken sharp tooth opposite the unilateral tongue lesion.

Table I shows the age, the CDC-group, the T-lymphocyte subsets in blood, and the pharmacologic treatment of the 14 HIV-seropositive patients at the time of the tongue biopsy, and also the occurrence in the biopsy specimen of EBV and/or fungal organisms. Patients F, G and H were classified as CDC-group IVC-2 for reasons other than HL. The mean age was 33.5 years, range 22–49 years. All 5 patients classified as CDC-group II or III had CD4+ cell counts within normal range, and a normal or slightly increased number of CD8+ cells. All 9 patients

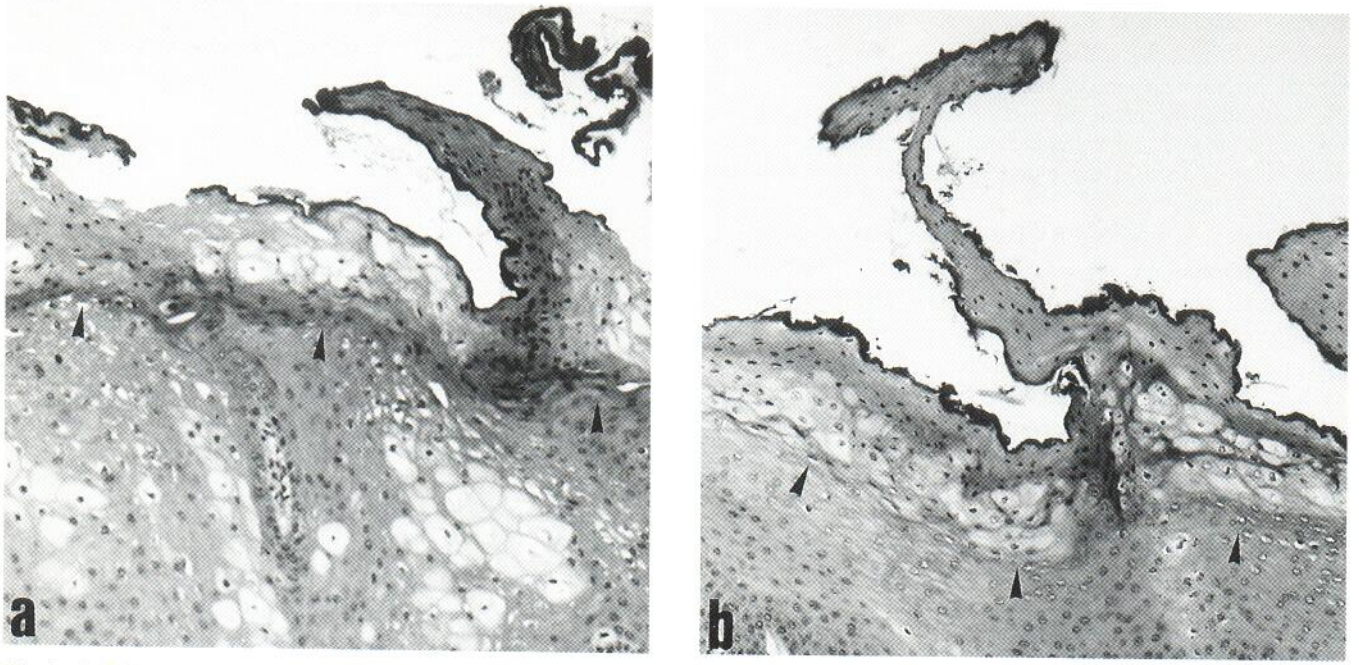


Fig. 3a, b. Biting lesions. (*a*) Bucca. (*b*) Tongue border. *Arrow-heads* indicate the line of demarcation.

classified as CDC-group IV had CD4+ cell counts below normal, and 6 of them had extremely low values. Two of these patients also had extremely low CD8+ cell counts. Of the remaining 7 patients, 2 had increased and 5 had normal CD8+ cell values. Since October 1990 7 patients have died, 6 due to AIDS; one patient committed suicide.

Prevalence of EBV and fungal infections

In HIV-seropositive patients the presence of EBV was proven by in situ hybridization (Fig. 4f) in 6 specimens (42.9%) and fungus infection (Fig. 4c) in 7 (50%), 6 candidiasis and 1 probably aspergillosis (histopathologically *Aspergillus*, but not verified by culture). Three patients (21.4%) were infected with both fungus and EBV. In 4 biopsy specimens (28.6%) neither fungus nor EBV was found.

EBV was not present in autopsy material or in biopsy specimens taken from biting lesions. In one of the tongues taken at autopsy a sparse amount of *candida* hyphae without any reaction in the tissue was observed. Fungal organisms were not found in samples taken from biting lesions.

Histology of the normal tongue

In our material the pieces taken from normal tongues, and cut transversely at right angles to the tongue border, included a part of the dorsal aspect, the whole border zone and a part of the ventral aspect. We found that the mucosa of the dorsal aspect had a thick and very irregular epithelium displaying filiform papillae cut in various directions. Often the cornified "hairs" were massively covered with bacteria. The squamous cell layer largely consisted of light cells, sometimes from the surface to the dark basal cell layer (Fig. 2a). There were also areas of ballooned and vacuolated cells (Fig. 2e). The upper part of the border zone had a thinner epithelium and a less irregular surface, but contained a few scattered rudimentary filiform papillae with one or two cornified "hairs". Towards the ventral aspect, the epithelium grew successively thinner and the surface was smooth. With the exception of the scattered filiform papilla "hairs", the epithelium of the border zone was non-keratinizing (Fig. 2b–d).

Histopathology of biting lesions

The epithelium could be divided in a lower and an upper compartment. The lower part consisted of an essentially normal epithelium with areas of ballooned and vacuolated cells, the upper part of a vacuolated or parakeratotic epithelium, distorted by biting or chewing, and with a frayed and often bacteria-coated surface displaying hair-like projections (Fig. 3a, b). The connective tissue contained no or very few lymphocytes.

Histopathology of macroscopically suspect HL lesions in 14 HIV-seropositive patients

The thickness of the epithelium varied from markedly thick (Fig. 4a) in the majority of cases to a rather thin configuration in the remaining few cases (Fig. 4d). In 4 of the cases with a thick epithelium, rudimentary, filiform papillae or papilla "hairs" covered with bacteria could easily be identified (Fig. 4a, b). In several specimens also a single fungiform papilla was observed (Fig. 4a). In all cases the epithelium was keratinizing in areas or in the whole specimen and displayed more or less marked hyper- and parakeratosis (Fig. 4c, d). The most conspicuous hyper- and parakeratosis was seen in case L, infected with *Candida* (Fig. 4c). In some specimens without *Candida* infection, the surface consisted of a thick layer of light vacuolated cells apparently ready to desquamate (Fig. 4a, b). With the exception of case B there were, in spite of fungus infection, no inflammatory cell infiltrates neither in the epithelium nor in the subepithelial connective tissue. In case B there were spongiotic pustules with a rich amount of neutrophils in the upper part of the epithelium, and a dense inflammatory cell infiltrate in the *lamina propria.* These are the common findings in acute muco-

cutaneous candidiasis in immunocompetent individuals. In only one case (A) was the surface partly covered with bacteria. In none of the cases the kind of "hairs" seen in biting lesions were observed; however, often the surface consisted of a band or a row of vacuolated and ballooned cells underneath an eosinophilic layer as seen in the biting lesion between the "hairs" (Figs. 4e, 3a, b).

No differences between EBV-positive and EBV-negative epithelium, indicating the presence of viral inclusion bodies in the former, were observed (Fig. 4b, e). In EBV-infected specimens the affected cells were located in the uppermost third of the epithelium and formed a continuous band (Fig. 4f), or multiple patches. Both non-vacuolated and vacuolated cells were involved. Changes typical of herpes virus or cytomegalovirus infections were not seen.

DISCUSSION

Histopathologic findings

Our investigation of the normal tongue mucosa shows that there is no distinct boundary line between the dorsal aspect and the border zone. Thus the configuration and thickness of the epithelium in biopsy specimens from the border zone vary depending on from which part of the border zone the biopsy is taken (Fig. 2a–c). This explains the differences of the histopathologic pattern of HL described by various authors (15), the differences between the macroscopic and microscopic appearance of lesions on the border zone and on the ventral aspect of the tongue (14), and also the difference in the frequency of "hairs" (14–16). Furthermore, we found that a layer of bacteria coating the epithelial surface was common on filiform papilla "hairs" in material from the normal tongue mucosa. Like previous investigators (5), we also found bacteria covering the surface to be a common occurrence in biting lesions (Fig. 3a, b). Therefore this phenomenon cannot be regarded as a significant feature of HL.

Various-sized areas of ballooned and vacuolated cells at different levels were common in the epithelium of the normal tongue, and in the epithelium of biting lesions, as well as in the epithelium of material taken from lesions on the tongue border in the HIV-seropositive patients, irrespective of the presence or absence of EBV-DNA (Figs. 2d, e, 3a, b, 4b, e, f). We therefore conclude that the presence of ballooned and vacuolated cells is as normal in the tongue mucosa as it is in the buccal mucosa and can thus be found in a high percentage also in biopsy specimens from white lesions of a variety of causes on the tongue. This is in accordance with the findings of Syrjänen et al. (9) and with those of Eversole et al. (13). Moreover, in contrast to Fowler et al. (17) and Fernández et al. (15) we have found no light microscopic indication of intranuclear virus inclusion bodies, either in vacuolated or non-vacuolated cells. Thus at present there is no evidence that EBV replication in oral epithelium is associated with special epithelial changes.

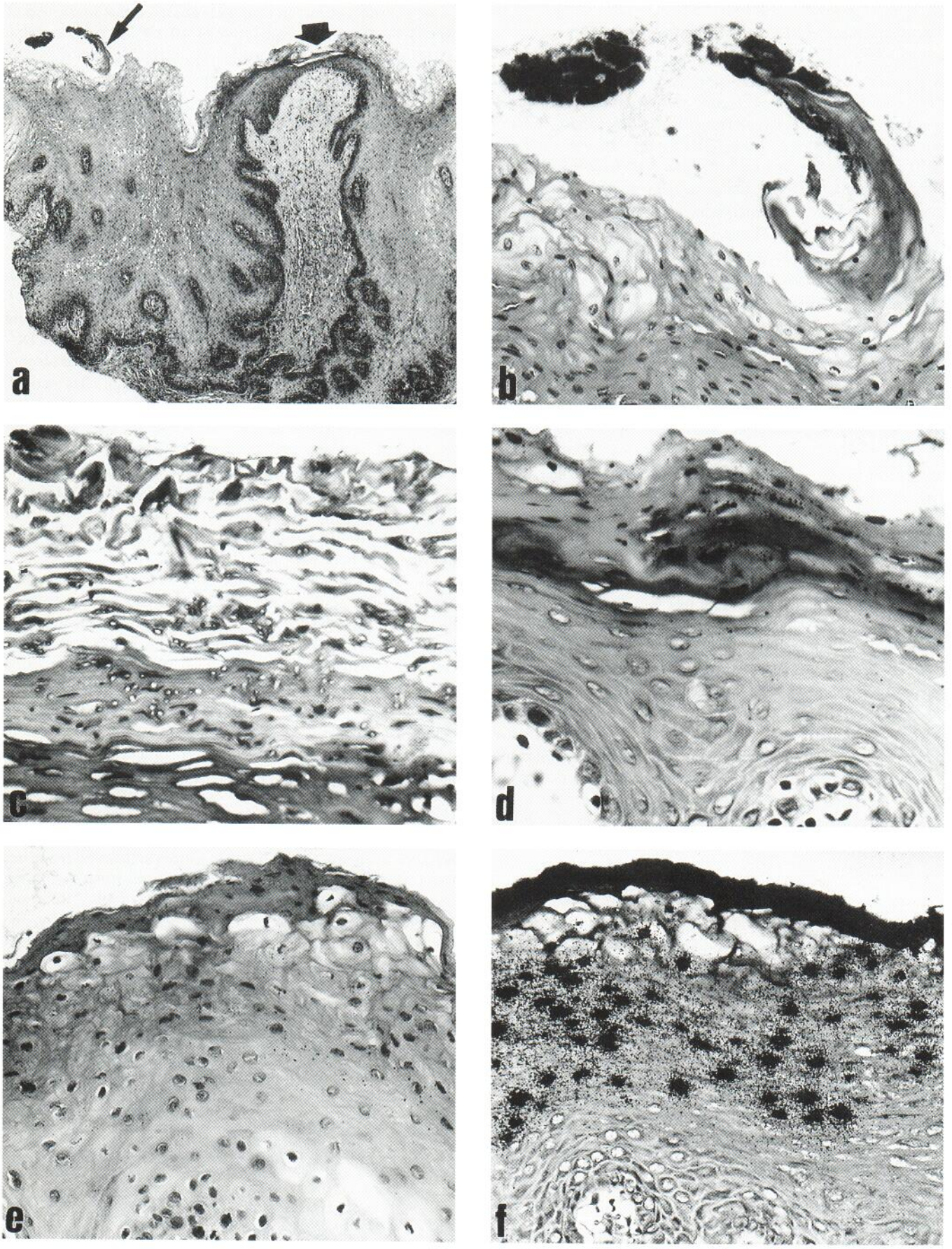
A typical biting lesion has hair-like projections (5). However, these "hairs" do not have the organized structure of the filiform papilla "hairs". Thus, a "hairy" histologic appearance in biopsy specimens from white lesions on the tongue border could be attributed to either a normal phenomenon or to biting or chewing (Figs. 2d, 3a, b, 4a, b). In our opinion the mucosal folds on the lateral borders of the tongue, described by Andersen et al. (7), form the basis of the macroscopically corrugated appearance of HL lesions.

The only consistent histopathologic finding in all clinically suspect HL lesions was a keratinizing epithelium, entirely or in areas hyper- and parakeratotic. In 4 cases (A, D, E, J) keratinization was found without evidence of viral or fungal infection. Patient A reported that he had had the habit of biting his buccal mucosa and his tongue since childhood. In this case the bilateral tongue and buccal lesions were all considered to be caused by biting. In patient E a tooth with sharp edges was located close to the suspected HL lesion. After treatment of the tooth the lesion disappeared. In patients D and J no obvious cause was found. Keratinization with orto- or parakeratosis is presumably the only histopathologic change necessary for a white unremovable lesion to appear on the oral mucosa.

The significance of fungal infections as the cause of unremovable white plaques on the tongue

In clinically suspected HL lesions the occurrence of *Candida* has been proven in up to 100% of the cases (1, 13, 15, 21). In order to sift the wheat from the chaff a course of so called adequate antifungal treatment was proposed. If the lesion did not disappear after this treatment it was accepted as HL. However, *Candida* hyphae have been found in a high percentage in biopsy specimens also after antifungal treatment (14, 16). It seems obvious that this is not an adequate method to exclude fungus infection as the cause of the lesion. In our material, 7 patients suffered from fungal infections, 2 of them being in the middle of treatment with one or several antifungal drugs. In the experience of one of us (E.L.), resistance to conventional antifungal drugs is a common phenomenon in patients with ARC or AIDS. Also, in spite of a successful treatment, it may take some time for the epithelium to return to normal. In all our cases with fungal infections the epithelium was hyper- and parakeratotic. If the fungal infection had a patchy distribution the hyper- and parakeratosis were restricted to these areas. We conclude that fungal infection (mostly candidiasis) is a major cause of unremovable patches on the tongue mucosa.

Fig. 4a–f. Biopsy specimens from patients D, L, E and K (*a*) D. The epithelium is thick and has a rudimentary filiform papilla with a "hair" (*thin arrow*) and a fungiform papilla (*thick arrow*). There is a thick layer of vacuolated and parakeratotic epithelial cells ready to desquamate. (*b*) Close-up of the bacteria coated "hair". (*c*) L. The uppermost part of a vacuolated epithelium with a very thick parakeratotic horny layer permeated with *Candida* hyphae. (*d*) E. The epithelium is thin, but keratinizing, and has a thick and parakeratotic horny layer. (*e*) K. The upper part of a thick and keratinizing epithelium. Below the horny layer is a row of vacuolated cells. (*f*) The same specimen as in (*e*). EBV-DNA is visualized by the in situ hybridization technique. Autoradiographic silver grains represent replicating virus. Only cells in the upper half of the epithelium, and mostly non-vacuolated cells, are affected.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The significance of EBV infection as the cause of unremovable white plaques on the tongue

A productive EBV infection, proven by in situ hybridization techniques or by means of electron microscopic investigations, is very common in biopsy specimens from HL lesions in HIV-seropositive patients (2, 15, 17, 20, 21). It is known since the 1970's that EBV can be found in saliva in up to 20% of healthy and asymptomatic EBV-seropositive individuals and in 50% or more of seropositive patients treated with immunosuppressive drugs, though it was not known from which kind of cells the virus originated (27). In 1984 Sixbey et al. (27), by using throat washing to collect material, found that the epithelial cells of the oropharyngeal mucosa contained productive EBV. Also in 1988 Alsip et al. (28) found that a significant number of both asymptomatic HIV-seropositive persons and patients with ARC and AIDS had detectable EBV-DNA in the oropharyngeal secretion, and that the concentration of EBV-DNA correlated with the increasing level of immunodeficiency. In 1991, Näher together with, among others, Greenspan and Greenspan (29) investigated the prevalence of EBV in the epithelial cells of the tongue mucosa in 11 HIV-seropositive patients with HL, and in 32 HIV-seropositive patients without HL. They found that all the 11 patients with HL were EBV-infected, but also 19/32 of the HIV-seropositive patients without HL. Moreover, statistical analysis revealed that the presence of EBV was significantly correlated with the clinical status of the HIV-infected persons, whereas HL was not. The finding that the presence of EBV is correlated with the clinical status of the HIV-infected patient is in accordance with the investigation of oropharyngeal secretion made by Alsip et al. (28), and with the findings in our material, where all EBV-positive samples but one came from patients classified as CDC-group IV. This strongly emphasizes the seriousness of productive EBV infection in HIV-seropositive patients. Lesions of HL are reported to disappear following anti-herpes virus treatment but reappear after cessation of therapy, pointing towards EBV as a possible cause of the lesion (30). However, as has been shown by Näher et al. (29), a productive EBV infection can even exist without any clinical manifestations.

The significance of unremovable white plaques as a prognostic marker of AIDS

Since 1984 Greenspan and co-workers have emphatically stressed the importance of HL as a herald of AIDS (1, 3, 22, 23), and consequently, in 1986, patients with HL were included in the CDC-group IV-2 (4). However, during the following years both their statements and their results have been somewhat contradictory. Thus in 1987, they found HL to represent a frequent and early clinical sign of exposure to HIV (14), excluding the possibility of HL being a herald of AIDS. In 1989, Green together with the Greenspans and de Souza (12) during a period of three years noticed 16 persons, HIV-seronegative or not at risk of HIV-infection, with clinically and histopathologically typical lesions of HL, in which the presence of EBV could not be proven. In 1991, they found, together with others (29), as already mentioned above, that the presence of EBV significantly correlated with the patients' clinical status, but not HL. Furthermore, in 1991 Greenspan et al. (22) observed that in a group of 198 HIV-seropositive patients with HL, 23 did not develop AIDS during an observation time of nearly 6 years.

Table I shows that our patients represented CDC-groups II, III and IV; thus the unremovable white plaques did not reflect the clinical status of the patients. Nor was the presence or absence of EBV and fungus infections reliable indicators, though mostly these kinds of infections were found in CDC-group IV. In some cases negative results could have been due to treatment; however, only 1 out of 3 patients who had got antiviral or antifungal treatment was EBV-negative or did not display fungal infection, respectively. The best correlation was found between CDC groups and CD4+ cell counts in peripheral blood. This is in agreement with the investigation of among others Böttiger et al. (26), who investigated 98 HIV-infected hemophiliacs and found a successively decreasing number of CD4+ cells from CDC-group II to CDC-group IV. In our patient B, a drug and alcohol abuser, candidiasis was proven in spite of a high CD4+ count; however, in contrast to all other biopsy specimens with fungal infection, the histopathologic investigation showed a conspicuous inflammatory cell infiltrate. We therefore consider this *Candida* infection comparable to a *Candida* infection in a person with a normal immune defence. Patient C, HIV-infected only for 18 months, is more puzzling. He was classified as CDC-group III (PGL), and had a high CD4+ count, but was infected with both EBV and *Candida*.

We agree with Greenspan and other investigators that white unremovable plaques on the tongue in an HIV-seropositive patient should alert the doctor or the dentist and should lead up to a thorough investigation of the patient including biopsy and CD4+ cell counts. However, we think it is equally important to be aware that white unremovable plaques on the tongue borders both in HIV-seropositive and HIV-seronegative persons may be completely harmless, as shown in our investigation and also in other reports (11, 12), and that it is important not to alarm the patient unduly.

Conclusions

The so called hairy leukoplakia in HIV-positive patients does not have a specific histopathologic pattern and can be the manifestation of e.g. fungus infections, mechanical irritation (biting, chewing or bad fillings), and possibly also of EBV infection. Therefore, in our opinion, this kind of lesion should not be considered a clinical entity. Replicating EBV is found in a high percentage of the lesions but can also be found in the epithelium of the oral mucosa without any macroscopically observable changes. In our investigation the presence of hairy leukoplakia did not reflect the clinical status of the patient. It appears that low CD4+ counts, candidiasis and EBV-infection are parallel markers of increasing immunodeficiency.

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