Mini review

Epstein–Barr virus in oral diseases

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Epstein–Barr virus (EBV), a B-lymphotropic gamma-herpesvirus, causes infectious mononucleosis and oral hairy leukoplakia, and is associated with various types of lymphoid and epithelial malignancies. Saliva is the main vehicle for EBV transmission from individual to individual. Recent studies have also implicated EBV in the pathogenesis of advanced types of periodontal disease. EBV DNA is detected in 60–80% of aggressive periodontitis lesions and in 15–20% of gingivitis lesions or normal periodontal sites. The periodontal presence of EBV is associated with an elevated occurrence of periodontopathic anaerobic bacteria. Moreover, EBV active infection occurs in ≈ 70% of symptomatic and large-size periapical lesions. EBV and cytomegalovirus often co-exist in marginal and apical periodontitis. Periodontal therapy can markedly suppress the EBV load in periodontal pockets as well as in saliva, which has the potential to reduce the risk of viral transmission between close individuals. EBV proteins up-regulate cytokines and growth factors, which seem to play a central role in the proliferative response of tongue epithelial cells in oral hairy leukoplakia and in the cell-transformation process of EBV-associated malignancies. Further research is needed to identify the full range of EBV-related diseases in the human oral cavity.

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In 1964, Epstein, Achong & Barr determined the causative agent of Burkitt's lymphoma to be a previously unknown member of the herpes family of viruses, later named the Epstein–Barr virus (EBV) (1). EBV was the first human virus to be assigned oncogenic potential. In 1968, EBV was demonstrated to be the major etiological agent of infectious mononucleosis (2). Since then, EBV has been implicated in a wide variety of malignant and benign tumors as well as in classic infectious diseases (3). A general introduction to the pathobiology of EBV can be found in *Fields Virology* (4,5).

EBV belongs to the Herpesviridae family, the gamma subfamily, and the Lymphocryptovirus genus. The EBV virion is composed of a linear, double-stranded DNA core contained within an icosahedral nucleocapsid of about 100 nm in diameter, which is surrounded by a proteinaceous tegument and a lipid bilayer envelope derived from the inner nuclear membrane of the host cell. Two genetically different forms of EBV, termed EBV type 1 and EBV type 2 (also known as type A and type B), are distinguished by allelic polymorphisms in the latent-cycle genes encoding the nuclear antigens 2 and 3 (EBNA 2 and EBNA 3, respectively) (6,7). EBV type 1 strains predominate in most western countries, whereas both EBV types are common in sub-Saharan Africa and Papua New Guinea (8). Both EBV types can simultaneously infect the same person. EBV type 1 is more potent than type 2 in achieving B-lymphocyte transformation *in vitro* (9), but both EBV types can act as human pathogens. The ensuing discussion relates primarily to EBV type 1 and the diseases caused by it, but the information is generally applicable also to EBV type 2.

EBV encodes almost 100 different antigens during the viral active phase, which involves the sequential expression of immediate early (IE), early (E) and late (L) proteins, and nine antigens in the latent phase, including nuclear antigens (EBNAs 1–6) and latent membrane proteins (LMPs 1–3). *In vitro*, EBV latent antigens are able to transform immunoglobulin-secreting B lymphocytes into immortal, perpetually dividing, lymphoblasts. EBV LMP-1, which is an integral membrane protein, seems to comprise the most important oncoprotein in mediating EBV-related malignant cell transformation. *In vitro*, LMP-1 mimics receptors of the tumor necrosis factor receptor superfamily (TRAFs) and activates numerous signaling pathways, including nuclear factor-kappaB (NF-κB) c-Rel, p38, c-Jun N-terminal kinase (JNK) and

phosphatidylinositol 3-kinase (PI3K)/ Akt (10). Perhaps because of the *in vivo* expression of LMP-1, these pathways are also activated in EBV-associated malignancies (10). Phylogenetically distinct LMP-1 variants can distinguish EBV oral pathogenic strains and have been used to study EBV transmission and persistence (11).

Despite the inflammatory and growth-transforming inducing capabilities of EBV, most infected individuals control the virus efficiently and remain free of EBV-associated diseases. As time is required for antigen-specific immune responses to develop and expand, the innate immune system, such as cytokines and natural killer (NK) cells, are important in the early phase of an EBV infectious process (12). In the phase of acquired immunity, EBV-specific CD3(+) CD8(+) cytotoxic T lymphocytes constitute the key defense cells against lytic EBV infections. Cytotoxic T lymphocytes control EBV infection by recognizing short peptides derived from the intracellular breakdown of viral proteins and presented at the infected cell surface by major histocompatibility complex (MHC) class I molecules. All EBNAs (except EBNA-1) and LMPs are target molecules for EBV-specific cytotoxic T lymphocytes. To circumvent vigorous T-lymphocyte-mediated immune responses, EBV uses latency in memory B lymphocytes to persist for the lifetime of the host. EBV-associated acute disease in pre-infected individuals occurs as a consequence of a decreased immune response and successive reactivation of the virus.

The life cycle of EBV involves two compartments: the peripheral blood and the oral cavity. EBV resides in B lymphocytes. Latently infected memory B lymphocytes circulate in the peripheral blood and are believed to constitute the main reservoir for EBV persistence. Permissively EBV-infected B lymphocytes in periodontal (13), and perhaps also tonsillar (14), tissues play a major role in the egress of virions into saliva.

EBV infects almost everyone in developing countries and 80–95% of adults in developed countries. In the USA, about 50% of all 5-year-old children, and nearly 95% of adults, harbor EBV. Periodic reactivation of EBV results in the shedding of virions into saliva, and asymptomatic carriers commonly spread the virus to uninfected individuals via salivary transfer (15). In Japan, EBV DNA was detected in 90% of throat washings from healthy adults (21–57 years old) and in 38% of saliva from healthy children (0–6 years old) (16). Salivary transmission of infectious EBV takes place through intimate oral contact or, possibly, by salivary residues left on cups, food, toys, or other objects. An EBV infection can also arise from transplanted bone marrow, kidneys, livers or hearts.

Infection with EBV in younger children is usually asymptomatic or mild. Clinical disease generally arises when primary infection occurs in adolescence or adulthood. EBV causes at least 90% of cases of infectious mononucleosis. EBV-induced mononucleosis is a generally self-limiting infection characterized by fever, pharyngitis, lymphadenopathy, hepatosplenomegaly, and malaise. Teenagers and young adults in their early twenties acquire mononucleosis (the 'kissing disease') by intimate contact with an EBV-infected person, with 1–3% of all college students in the USA affected each year. Persistently high EBV loads in saliva during convalescence from mononucleosis poses an infectious risk for close acquaintances (17). Antiviral drugs in placebo-controlled trials generally fail to provide benefits for patients with uncomplicated infectious mononucleosis, probably because the disease is mainly caused by immune responses to EBV-infected activated B lymphocytes rather than by direct viral replication (3).

The ability of EBV latent infections to transform B lymphocytes and epithelial cells can give rise to tumors in lymphoid tissue and in naso- and oropharyngeal epithelial tissue (18). Patients with EBV-associated lymphomas and carcinomas reveal the presence of EBV DNA in cancer cells as well as high antibody levels against EBV antigens. EBV shows a close relationship with Burkitt's lymphoma, Hodgkin's lymphoma, X-linked lymphoproliferative syndrome, posttransplant B-lymphocyte lymphoma, and brain lymphoma in patients with acquired immune-deficiency syndrome (AIDS), and with undifferentiated nasopharyngeal carcinoma, salivary gland lymphoepithelial carcinoma, and various types of gastric carcinoma. Burkitt's lymphoma is a B-lymphocyte tumor that affects approximately eight in every 100,000 children in equatorial Africa and Papua New Guinea (5). Allogeneic stem cell transplantation can evoke an active EBV infection, which may progress to EBV-associated lymphoproliferative disorders (19). Nasopharyngeal carcinoma is confined to the nasal passages and throat, and is endemic among Cantonese Chinese people in southern China, but is also prevalent among natives of southeastern Asia, Arabs in North Africa, and Inuits in Alaska and Greenland (20).

In the past decade, herpesviruses have been implicated in the etiopathogenesis of destructive periodontal diseases (21). It has been theorized that an active herpesvirus infection in the periodontium, by inducing immunosuppression, lessens the efficient control of periodontopathic bacteria, which then become able to initiate or expand the breakdown of tooth-supportive tissues (21). A recent review discussed the importance of human cytomegalovirus (HCMV) in periodontal disease (22). Although HCMV may be the more important periodontopathic herpesvirus, EBV may contribute unique pathogenic properties to the development of periodontitis. The present article highlights findings that associate EBV with severe types of marginal and apical periodontal disease, oral hairy leukoplakia, and various types of malignancies in the human mouth. It is assumed that the ability of EBV to express cytopathogenic effects, immune evasion, immunopathogenicity, latency, reactivation from latency, and tissue tropism is of relevance for the pathogenesis of EBV-related oral diseases.

Periodontal diseases

Gingivitis and periodontitis are the two major periodontal diseases in humans. Chronic gingivitis connotes inflammation of the gingiva with no destruction

of the underlying alveolar bone. Dental plaque bacteria cause gingivitis, and proper dental hygiene can prevent the disease onset or reverse the course of existing disease. Periodontitis is characterized by loss of alveolar bone and periodontal ligament fibers attaching the tooth to the bone. Some types of periodontitis are self-limiting and will spontaneously burn out after a relatively brief period of activity, while other disease types undertake a prolonged destructive course that may eventually lead to tooth mobility and loss of teeth. Mild-to-moderate periodontitis afflicts 20–50%, and aggressive periodontitis 5–15%, of adults in North American (23) and western European (24) countries. The more severe forms of periodontal disease occur with increased frequency in populations of developing countries and among individuals of low socioeconomic status (25).

Table 1 lists the occurrence of EBV in periodontitis lesions from various parts of the world. All published studies describe a significantly higher occurrence of EBV DNA in aggressive periodontitis than in gingivitis or nonprogressing periodontitis. The reasons for variation in EBV occurrence among studies may include differing EBV detection techniques, dissimilar periodontitis disease states studied, and true geographic variation in EBV prevalence. Although EBV type 1 is most commonly identified in aggressive and chronic periodontitis lesions, both EBV-1 and EBV-2 can occur in periodontitis lesions (34,39). In Chinese patients, Wu *et al.* (39) detected EBV-1 in 29%, 17% and 15%, and EBV-2 in 8%, 3% and 0%, of chronic perio-

dontitis, gingivitis and healthy periodontal sites, respectively. In human immunodeficiency virus (HIV)-infected patients, who are frequently infected with EBV-2 (41), EBV-2 DNA has been identified in as many as 57% of the periodontitis lesions studied (42). EBV has also been associated with rapid gingival tissue destruction in an HIV-infected patient (43), and with hyperplastic and cyanotic gingiva in two cardiac transplantation patients with a history of cyclosporine use (44).

Table 2 shows EBV–HCMV periodontal co-infection to be associated with particularly severe types of periodontal disease, perhaps because of the potential of an active herpesvirus infection to *trans*activate other co-resident herpesvirus species (46). Simultaneous EBV–HCMC replication may significantly expand the

Table 1. Epstein–Barr virus (EBV) DNA in periodontal sites

Study[a]	Country	Periodontal status	Occurrence of Epstein–Barr virus[b]
Contreras *et al.* (26)	USA	Advanced chronic periodontitis	79% (chronic periodontitis)
			27% (periodontal health/slight gingivitis)
Ting *et al.* (27)	USA	Localized aggressive periodontitis	64% (aggressive periodontitis)
			18% (periodontal health)
Michalowicz *et al.* (28)	Jamaica	Localized periodontitis	33% (aggressive periodontitis)
			45% (incipient periodontitis)
			17% (periodontal health/gingivitis)
Kamma *et al.* (29)	Greece	Generalized periodontitis	44% (disease-active periodontitis)
			13% (disease-stable periodontitis)
Konstantinidis *et al.* (30)	Greece	Chronic periodontitis	56% (periodontal pockets > 7 mm)
			9% (periodontal pockets < 4 mm)
Saygun *et al.* (31)	Turkey	Generalized periodontitis	72% (aggressive periodontitis)
			6% (periodontal health)
Saygun *et al.* (32)	Turkey	Periodontal abscess	72% (periodontal abscess)
			0% (periodontal health)
Kubar *et al.* (33)	Turkey	Generalized periodontitis	89% (aggressive periodontitis)
			46% (chronic periodontitis)
Klemenc *et al.* (34)	Slovenia	Chronic periodontitis	44% (chronic periodontitis)
			0% (periodontal health)
Joseph *et al.* (35)	Romania	Gingiva of HIV-infected children	75% (EBV LMP-1 in gingiva of HIV-positive children)
			47% (EBV LMP-1 in gingiva of HIV-negative children)
Madinier *et al.* (36)[c]	France	Gingiva at the site of tooth extraction	40% (gingival papillae)
Ling *et al.* (37)	Taiwan	Chronic periodontitis	4% (chronic periodontitis)
Li *et al.* (38)	China	Chronic periodontitis	58% (disease-active periodontitis)
			23% (quiescent periodontitis)
			19% (gingivitis)
Wu *et al.* (39)	China	Chronic periodontitis	37% (chronic periodontitis)
			20% (gingivitis)
			15% (periodontal health)
Idesawa *et al.* (40)	Japan	Chronic periodontitis	49% (saliva of periodontitis patients)
			15% (saliva of periodontally healthy subjects)

[a] EBV was identified by means of conventional (end-point) or real-time polymerase chain reaction techniques.
[b] Most studies report on EBV type 1.
[c] EBV was identified by Southern blotting using a ^{32}P-radiolabelled DNA probe.
HIV, human immunodeficiency virus; LMP-1, latent membrane protein-1.

Table 2. Epstein–Barr virus (EBV) and human cytomegalovirus (HCMV) co-infection in periodontal disease

Study[a]	Periodontal disease	Percentage of aggressive periodontal lesions with EBV–HCMV co-infection	Percentage of stable periodontal sites with EBV-HCMV co-infection
Ting *et al.* (27)	Localized periodontitis in teenagers	60%	22%
Kamma *et al.* (29)	Aggressive periodontitis in young adults	28%	0%
Saygun *et al.* (32)	Periodontal abscess	56%	0%
Contreras *et al.* (45)	Acute necrotizing ulcerative gingivitis in children in Nigeria	36%	0%

[a] Viruses were identified by means of conventional (end-point) polymerase chain reaction techniques.

CD8-positive cytotoxic/suppressor T-lymphocyte subset and decrease the number of functional CD4 T lymphocytes, resulting in a wide-ranging suppression of antibacterial host defenses within the periodontium.

Herpesvirus model for periodontitis

EBV may cause periodontal disease as a direct result of virus infection and replication, or as a consequence of virally induced impairment of periodontal host defenses with heightened aggressiveness of resident bacterial pathogens (21). Similarly to herpesvirus medical infections, the early stages of EBV-associated periodontitis in immunologically naïve hosts may mainly comprise direct cytopathogenic events, whereas most clinical manifestations in immunocompetent individuals are secondary to cellular or humoral immune responses. This concept of periodontal disease pathogenesis seems to agree with a rapid rate of tissue destruction at the debut of periodontitis, and little or no further tissue breakdown at later 'burned-out' disease stages.

Herpesviruses may exert direct cytopathic effect on fibroblasts, keratinocytes, endothelial cells, inflammatory cells, and bone cells in the periodontium. Phagocytic and bactericidal capacities of periodontal neutrophils, cells of key importance in the periodontal defense (47), showed significant impairment in periodontitis patients revealing herpesvirus-like viral bodies in oral lymphocytes and epithelial cells, as compared with virus-negative persons (48). EBV infection and damage of the periodontal pocket epithelium may contribute to gingival bleeding, as suggested by a high prevalence of EBV DNA in periodontal sites exhibiting bleeding upon probing (29,34,40). However, as seen in localized aggressive periodontitis, EBV DNA can also be detected in periodontal sites with minimal bleeding (27).

The presence of EBV influences the T helper cell 1 (Th1)/T helper cell 2 (Th2) balance towards the production of Th1 cytokines and chemokines (49). Cytokines and chemokines regulate inflammatory responses and play important roles in the first line of defense against human herpesvirus infections, and also contribute significantly to the regulation of acquired immune responses, wound healing, and other biologic processes. An active EBV infection stimulates host cells to produce interleukin-1α, interleukin-1 receptor antagonist (interleukin-1Ra), interleukin-6, interleukin-8, interleukin-18, tumor necrosis factor (TNF)-α, interferon (IFN)-α/β and IFN-γ, monokine induced by IFN-γ (MIG), IFN-γ-inducible protein 10 (IP-10), and granulocyte–macrophage colony-stimulating factor (GM-CSF) (49). Pro-inflammatory cytokine and chemokine activities serve a positive biological goal by aiming to overcome infection or invasion by infectious agents. IFN-γ, TNF-α, and interleukin-6 exert particularly high antiviral activity. In the context of periodontitis, cytokines may also contribute to tissue destruction by initiating and sustaining the inflammatory infiltrate and by stimulating alveolar bone resorption (50). Pro-inflammatory cytokines tend to occur at elevated levels in active periodontitis sites, perhaps because of an ongoing herpesvirus infection (51). However, although the biologic activities of pro-inflammatory cytokines are consistent with the signs of periodontitis, no firm evidence exists to assign key importance to any particular cytokine in the etiopathogenesis of the disease.

Periodontal herpesvirus infections are associated with increased levels of periodontopathic bacteria. In adult periodontitis, the presence of EBV DNA is related to an elevated occurrence of *Porphyromonas gingivalis*, *Tannerella forsythia*, *Campylobacter* species and other periodontopathic bacteria (31,51,52). By perturbing inflammatory cells involved in the periodontal defense, a periodontal EBV infection may predispose to proliferation of periodontopathic bacteria, or may affect the adhesion potential of pathogenic bacteria to infected host cells. Sugano *et al.* (53) showed the mean proportion of salivary *P. gingivalis* to be 0.25% in EBV-positive and 0.02% (i.e. a 13-fold reduction) in EBV-negative periodontitis patients, and found *P. gingivalis* sonicate to increase EBV reactivation *in vitro* by five- to ninefold compared with the control. Sugano *et al.* (53) proposed the EBV–*P. gingivalis* interaction to be bidirectional, with *P. gingivalis* having the ability to induce EBV reactivation, and EBV reactivation having the potential to suppress host defenses and permitting overgrowth of *P. gingivalis*.

Herpesvirus-related periodontal disease may progress in a series of steps. Initially, bacterially induced gingivitis permits EBV-infected B lymphocytes to enter the periodontium. An activation of latent EBV in the periodontium may then occur spontaneously or as a result of a concurrent infection, fever, drugs, tissue trauma, emotional stress or other factors impairing the host immune defense. EBV activation takes place

during periods of inadequate EBV-restricted cellular cytotoxicity, causing an outgrowth of EBV-infected B lymphocytes and a release of tissue-damaging mediators. The abundance of B lymphocytes observed in some periodontitis lesions (54) may partly be caused by an EBV-mediated polyclonal B-lymphocyte activation (55). Established risk factors or indicators of periodontal disease, including HIV infection, psychosocial and physical stress, pregnancy, and hormonal changes (56), have the potential to reactivate herpesviruses, which may be an important reason for their pathogenic relationship with periodontal disease. An active herpesvirus infection may also impair periodontal neutrophils (48) and other host responses (57) of importance in controlling periodontitis-causing bacteria. Disease remission may take place after the establishment of an efficient herpesvirus control, which may require homing of herpesvirus-specific CD8$^+$ T lymphocytes to infected periodontal sites (58). The proposed periodontal disease model emphasizes the importance of reactivation of latent periodontal herpesviruses. EBV reactivation may occur relatively frequently in periodontal sites but, in most immunocompetent individuals with acquired EBV immunity, may not persist for a period long enough to cause clinical breakdown.

Treatment of EBV periodontal infection

The notion that EBV and other herpesviruses play a significant role in several types of severe periodontal disease has therapeutic implications. A new direction for preventing and treating periodontitis may focus upon controlling periodontopathic herpesviruses. Recent studies have shown that antimicrobial periodontal therapy can greatly reduce the herpesvirus load in the periodontium (32,59,60), probably because the persistence of periodontal herpesviruses depends on the continuous presence of infected inflammatory cells or a virus-mediated inhibition of apoptosis (61).

As the primary route of EBV transmission is through salivary exchange (5), and because EBV transmissibility may be directly proportional to the salivary EBV viral load, the oral source(s) of EBV need to be identified. A recent study showed that healthy salivary glands are unlikely to harbor EBV, and that tongue epithelial cells only infrequently support EBV replication (14). The finding that periodontal treatment can markedly reduce EBV counts in saliva, sometimes to undetectable levels (40,60), suggests that inflamed periodontal sites constitute the major source of salivary EBV. Following periodontal therapy, Saygun *et al.* (60) found a decrease in EBV DNA counts in periodontal pockets by 5.7-fold and in saliva by 12.9-fold. Idesawa *et al.* (40) found the average EBV counts per ml of saliva to decrease from 946,000 to 9010 after periodontal therapy, and six of 11 (54%) patients showing salivary EBV pre-treatment did not reveal the virus post-treatment. Several patients in the studies of Saygun *et al.* (60) and Idesawa *et al.* (40) exhibited gingival inflammation post-treatment; more diligent antigingivitis measures may have decreased the salivary EBV counts even further (62). The potential of periodontal therapy to decrease the EBV salivary load of a carrier and thereby to reduce or prevent EBV transmission and EBV-related oral and nonoral diseases among close acquaintances may have significant public health implications.

Endodontic diseases

Pulpitis and apical periodontitis are the two major endodontic diseases. Pulpitis denotes inflammation of the dental pulp, which usually evolves as a sequel to a dental caries lesion penetrating into the pulp, or to a traumatic injury to the teeth. Apical periodontitis is an extension of pulpitis and connotes a destruction of the bone around the apex of the root. Pain is a common feature of pulpal and periapical inflammation.

Endodontic inflammation can be initiated and sustained by a variety of infectious agents and is mediated by both cellular components (i.e. macrophages, lymphocytes, and leukocytes) and molecular components (including cytokines and chemokines), many of which possess pro- or anti-inflammatory properties, with potential harmful or beneficial effects (63,64). Bacteria in acute periapical abscesses or in root canals with asymptomatic periapical lesions include the anaerobic species *P. gingivalis*, *Porphyromonas endodontalis*, *Prevotella intermedia*, *Fusobacterium nucleatum*, *Treponema denticola*, *T. forsythia*, *Dialister pneumosintes*, and *Peptostreptococcus micros* (65). It is assumed that endodontopathic bacteria, together with a diminished host resistance, are responsible for flare-ups of periapical lesions.

Herpesviruses seem to participate in the pathogenesis of symptomatic periapical lesions (Table 3). Symptomatic and large-size periapical lesions exhibit a significantly higher frequency of EBV and HCMV active infections than asymptomatic lesions of similar radiographic size, or of small-size lesions (68,69). Although HCMV appears to be the more important endodontopathic herpesvirus, EBV

Table 3. Epstein–Barr virus (EBV) and human cytomegalovirus (HCMV) in symptomatic and asymptomatic periapical lesions

Herpesvirus	Number (%) of symptomatic lesions		Number (%) of asymptomatic lesions
	$(n = 25)^a$	$(n = 12)^b$	$(n = 19)^a$
HCMV alone	6 (24%)	3 (25%)	2 (11%)
EBV alone	0	4 (33%)	0
HCMV and EBV	19 (76%)	4 (33%)	5 (26%)
Neither HCMV nor EBV	0	1 (8%)	12 (63%)

[a] Adult patients. Viruses were identified by means of a reverse transcription–polymerase chain reaction technique. Difference between herpesviruses in symptomatic and asymptomatic periapical lesions: $p < 0.0001$ (chi-squared test). Adapted from Slots *et al.* (66).
[b] Children with primary teeth. Adapted from Yildirim *et al.* (67).

and HCMV are often co-residents in severe periapical disease (66). TNF-α, interleukin-1β, interleukin-6, chemokines, interferons, and other multifunctional mediators have the potential to propagate states of hyperalgesia (70) and bone resorption (71). An active herpesvirus infection may cause the release of hyperalgesic and bone-resorping mediators from periapical inflammatory and connective tissue cells. Also, in a vicious circle, a vigorous release of cytokines may activate latent herpesviruses and in so doing may further aggravate periapical pathosis. Moreover, similarly to marginal periodontitis, herpesvirus-induced immune impairment may cause increased growth of endodontic gram-negative anaerobic bacteria (72), whose lipopolysaccharide may further up-regulate Th1 cytokine release from mammalian cells (63) and act synergistically with herpesviruses in transcription of the interleukin-1β gene (73).

Oral hairy leukoplakia

Oral hairy leukoplakia (OHL) was first described in the 1980s (74,75). OHL almost always presents on the lateral and dorsolateral parts of the tongue as white vertical folds or ridges that cannot be scraped off. Histologically, OHL shows vacuolated epithelial cells (koilocyte-like), and little or no inflammatory infiltrate in the underlying connective tissue. OHL is almost exclusively seen in immunocompromised patients, particularly in HIV-infected individuals in whom OHL may serve as an indicator of progression to AIDS. OHL may occasionally appear in immunodeficient patients receiving cancer chemotherapy or organ transplantation, or having leukemia. Antiherpesviral therapy with acyclovir may lead to remission or disappearance of OHL. However, OHL is benign and causes no symptoms or medical/dental problems, and usually does not require treatment.

EBV productive and nonproductive infection of tongue epithelial tissue in HIV-infected individuals is closely associated with OHL (74). In immunocompetent individuals, EBV

Table 4. Epstein–Barr virus (EBV)-related tumors in the oral cavity

Tumor	Location	Comment	Study
Lymphoproliferative disorder-tumor	Gingiva	Following kidney transplantation	Le Meur *et al.* (89)
Non-Hodgkin lymphoma	Gingiva, alveolar mucosa	HIV-infected individuals	Iameroon *et al.* (90)
Natural killer/T-cell lymphoma	Gingiva, the palate, the tongue	Poor prognosis	Cho *et al.* (91), Yin *et al.* (92)
Follicular lymphoid hyperplasia	Cheek	Strong expression of EBV-encoded RNA	Kojima *et al.* (93)
Oral lymphomas	One lymphoma arose at a site of previous oral hairy leukoplakia	HIV-infected patients. EBV-containing lymphomas were high-grade histological subtypes, including Burkitt's lymphoma	Palmer *et al.* (43), Gulley *et al.* (94)
Burkitt's lymphoma	Periapical lesions and other oral sites of Israeli patients	A 2-year survival rate of 62%	Ardekian *et al.* (95)
Oral lymphomas	Patients from Tanzania	70% of Burkitt's lymphomas showed EBV DNA	Syrjänen *et al.* (96)
Salivary gland lymphoepithelial carcinomas	Patients from Asian countries and Russia	EBV closely associated with the cancer	Wu *et al.* (97), Wang *et al.* (98), Saku *et al.* (99), Leung *et al.* (100)
Warthin's tumor	Salivary (parotid) glands	EBV associated with multiple/bilateral Warthin's tumors	**Positive EBV relationship:** Wang *et al.* (101), Santucci *et al.* (102). **No EBV relationship:** Laane *et al.* (103), van Heerden *et al.* (104)
Squamous cell carcinoma	Tongue/oropharyngeal sites	Co-infection with EBV and papillomavirus	**Positive EBV relationship:** Hermann *et al.* (105), Higa *et al.* (106), Szkaradkiewicz *et al.* (107). **No EBV relationship:** Cruz *et al.* (108)

HIV, human immunodeficiency virus.

possesses little or no ability to replicate in tongue epithelial cells (76). Sandvej *et al.* (77) suggested that OHL was caused by repeated direct infection of tongue superficial epithelial cells by EBV originating from saliva rather than by a latent EBV infection of basal epithelial cells. The source of the salivary EBV may be inflamed periodontal pockets in intimate contact with the lateral borders of the tongue. The possibility also exists that an active exchange of EBV strains takes place between infected peripheral blood lymphocytes and OHL (78).

Although the association of EBV with OHL has been firmly established, the precise role of EBV in causing OHL is less clear. OHL lesions show EBV-encoded LMP-1 (79) and nuclear antigen (EBNA)-2 (80) protein functions. LMP-1 and EBNA-2 can up-regulate cytokines and growth factors and make separate and complementary contributions to the survival of latently EBV-infected cells (81). EBV genes also encode homologues, similar to human counterparts, of the antiapoptotic B-cell leukemia-lymphoma 2 (Bcl-2) protein (82), cytokines and chemokine receptor-related G protein-coupled receptors (GPCRs) (83), the mitotic cyclin B_1 (84), and the anti-inflammatory interleukin-10 having the potential to enhance EBV productive replication (83). Cyclin B_1 and interleukin-10 are over-expressed in OHL lesions (84). Also, EBV LMP-1 up-regulates Bcl-2 (85), which is abundantly expressed in the upper layers of OHL lesions (86). It is assumed that the pathogenesis of OHL includes EBV gene products homologous to important human proteins and EBV-mediated cytokine release from tongue cells, the combined effect of which may be interference with cell cycle regulation, induction of immunosuppression, and inhibition of apoptosis.

EBV-related oral tumors

EBV latent infection is linked to the development of various lymphoid and epithelial human malignancies (87). The EBV-mediated disruption of cell-growth checkpoints relies to a great extent on a direct modulation of cytokine receptor signaling mechanisms and alterations in the expression levels of various cytokines. LMP-1 and other EBV latent proteins exert pleiotropic effects when expressed in cells, resulting in the up-regulation of cytokines (interleukin-6 and interleukin-8) and growth-activating factors (TRAF) with the ability to promote cell transformation, modulate immune responses, and induce antiapoptotic mechanisms. Table 4 lists some oral tumors that have been ascribed to EBV. The virus can be associated with aggressive types of oral tumors, especially in immunosuppressed patients (88). Most EBV-related oral tumors involve the tongue and the parotid gland, but some are located in the periodontium (Table 4). Research is needed to determine the extent to which EBV participates in oral tumorigenesis and to delineate the complex and varying interplay between the virus and the host cell environment in different types of oral cancer.

Concluding remarks

The present article suggests that EBV oral infections can contribute significantly to periodontal morbidity and even patient mortality. However, delineating the true significance of EBV in oral diseases will require longitudinal studies to provide information about the persistence and levels of strains, and about EBV-specific host responses that may lead to the development of disease or to the maintenance of an asymptomatic state. In particular, studies are needed to elucidate key elements of EBV periodontal infection, including periodontal tropism, persistence, and periodontopathic determinants of the virus, all of which are poorly understood. High-priority studies include the evaluation of emerging immunotherapeutic strategies to prevent or control EBV clinical infections in the human oral cavity.

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