

The role of viruses in oral disease

Bjørn Grinde^{1,*} and Ingar Olsen²

¹Department of Virology, Division of Infectious Disease Control, Norwegian Institute of Public Health, Oslo, Norway; ²Institute of Oral Biology, University of Oslo, Oslo, Norway

The focus has traditionally been on bacteria and fungi when discussing microbiological aspects of oral disease. Viruses are probably more involved in diseases associated with the oral cavity than has been previously thought. The role of several viruses in ulceration is well known, but viruses of the herpes family may play a role in periodontitis, and papillomaviruses are probably involved in oral cancer. This review offers a brief introduction to virology before discussing the role of the more relevant viruses in oral disease. As to clinical application, it is concluded that the anti-herpes medication may, in some cases, be relevant in treating periodontitis, while papillomavirus vaccine would be expected to decrease the prevalence of oral cancer.

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The oral cavity is home to a rich flora of bacteria; some 700 different species have so far been described (1). Viruses are by nature more difficult to detect, at least with traditional methods such as *in vitro* cultivation. However, the advent of the tools of molecular biology, particularly various PCR-based methods, has changed the situation. We can now readily detect not only bacteria that have not yet been cultivated, but also human viruses, which are indeed common in samples from the oral cavity. Consequently, it is important to know the role that viruses play in oral disease. This topic has recently been reviewed extensively (2).

While it has long been known that most of the bacteria associated with your body do not cause any harm, viruses have a more dubious reputation. The point probably reflects, at least partly, the fact that they have been more invisible to the clinician. Prior to the advent of PCR, people rarely looked for viruses except as suspects when diagnosing a particular clinical condition; and if a virus was found, it was typically assumed to be the cause of the condition. Recent investigations have shown that certain viruses are highly prevalent in the human body. Circoviruses, for example, are present in more than 90% of adults and may never cause any disease (3, 4); polyomaviruses are present, at least in some points in life, in a majority of the population, but are only rarely associated with any symptoms (5). Moreover, even viruses that are known to be capable of serious complications can be highly prevalent in people without any overt symptoms; examples include the herpesvirus family and enteroviruses. Thus, the mere presence of virus in a sample taken from diseased oral tissue is not sufficient to

implicate the virus in the pathological changes observed. The present review discusses the evidence for a direct viral involvement, and to what extent that should affect clinical practice in the form of treatment or preventive measures. The focus is on viruses that may cause clinical symptoms, as opposed to those that simply use an oral route of infection, or transmission, without manifesting themselves in the mouth; or viruses that affect oral health indirectly, for example, by causing immunodeficiency, such as HIV.

Clinical virology

The process of evolution has shaped viruses towards the same objective as other organisms, that is, survival and procreation. As with any obligate parasite, a winning strategy requires not just efficient replication, but also a means of transmission between hosts. Consequently it is not an optimal strategy to kill the host; in fact, it is generally preferable to reside in an active, implying not very sick, host. Unfortunately, evolution rarely forms optimal organisms.

One feature that distinguishes viruses is their extreme form of what in biology is referred to as 'r-selection'. The term is used for organisms that produce as much progeny as possible without bothering about quality assurance or putting resources into each one of them. This strategy allows the virus to have more sloppy machinery for RNA or DNA replication than any other cellular organisms; viruses can afford to make thousands of worthless copies of themselves for each competent viral particle. The point is a nightmare for clinicians, in that viruses relatively easily develop resistance to antiviral medication, and in

that they escape vaccines or the immunological memory of previous infections.

Although viruses as a rule of thumb are best served by having a healthy host, they can still cause serious disease. Three factors are of particular importance for the clinical outcome of a viral infection. For one, pathology depends on how advanced the relationship between virus and host is. Viruses that have a long evolutionary history with a particular host generally have evolved mechanisms to avoid unnecessary damage to their host, while viruses that recently jumped from one species to another have not had the chance to do so. Consequently, zoonotic viruses pose a particular threat to human health. The second factor is whether the virus is inclined to remain with the host for a long time, preferably throughout the life-span, or if it is more of a 'hit-and-run' virus. In the latter case there is obviously less selection as to restraining the virus from causing harm. The third factor is whether the virus requires particular behaviour of the host in order to be transmitted to another individual; coughing is perhaps the most obvious and well-known example. In Table 1, the various strategies and clinical symptoms are outlined schematically.

Viruses can infect any type of cell in human body, with the possible exception of erythrocytes; still the mouth has a particular significance. For one, the mouth offers a perfect entrance to a new host. We breathe, drink and eat every day; thus all that is required for the virus is a strategy that allows transmission either through air or through water/food. The former is typically cared for by the infected host when he coughs and thus sends out aerosols containing viral particles; the latter is reflected in transmission by the faecal-oral route, that is, the virus infects the guts and is thus released to the environment through the faeces. In order to pursue these strategies, viruses are not required to replicate in the mouth, and most viruses do not. For various reasons they prefer, respectively, the respiratory system or the intestines, consequently the concomitant disease is typically restricted to these organs.

In certain cases, however, replication in the mouth may be preferable.

Humans are not the only species that have developed a delight in kissing, but probably no other species are prone to engage in the exchange of sputum to the extent that we humans are. Not surprisingly, certain viruses apparently have adapted to this behaviour in their approach to the issue of transmission. In order to assure viral presence in sputum, it is preferable to replicate in cells lining the oral cavity and release a continuous stream of viral particles. Herpesviruses and papillomaviruses are among the viruses that presumably use this strategy. Obviously, replication in oral tissue places the oral cavity more at risk for clinical symptoms.

The herpesvirus family

Herpesviruses have a double-stranded DNA genome and are among the largest and most complex human viruses. There are eight members of the human herpesvirus (HHV) family (Table 2). The more common, as to oral health problems, are the two herpes simplex viruses (HSV-1 and -2), which cause recurrent herpetiform ulcerations referred to as cold sores. These ulcers typically occur on the lips, but the viruses can also cause similar lesions in the mucosa, such as in the case of gingivostomatitis. The mucosal affection is normally associated with a primary herpes infection in children, and is accompanied by bodily symptoms such as fever and malaise. Both HSV-1 and -2 may be involved in oral manifestations, although the latter is primarily associated with the genitals.

Epstein-Barr virus (EBV) and cytomegaloviruses (CMV) are present in the vast majority of adults, but in most cases probably without ever causing any overt disease. Both can, however, cause mononucleosis; EBV being responsible for most of the cases. Mononucleosis is also known as 'kissing disease', suggesting that the virus spread through direct mouth-to-mouth contact. The condition is common at puberty, and is considered a consequence of the host not having been in contact with

Table 1. Schematic presentation of clinical symptoms caused by viruses.

Rational	Symptoms/disease	Examples
Virally intended: 'hit-and-run' viruses	Coughing	Rhinoviruses, influenza
	Diarrhoea	Noro- and rotaviruses
	Bleeding	Ebola- and hantaviruses
Virally intended: chronic viruses	Blisters, soars, ulcers	Herpes- and enteroviruses
	Behavioural change, e.g. aggression	Rabies virus
Not in viral strategy	Cancer	Papilloma- and polyomaviruses
	Immunodeficiency	HIV
	Fever	Many viruses
	Inflammation and concomitant tissue damage	Many viruses
	Gross immunological overreaction and secondary infections	Pathogenic influenza

Table 2. Classification of human herpesviruses (HHV) and their associated diseases.

Type	Primary target cell	Oral affection	Other pathology
1. Herpes simplex virus-1	Mucoepithelial	Herpes ulcers	Genital ulcers
2. Herpes simplex virus-2	Mucoepithelial	Herpes ulcers	Genital ulcers
3. Varicella Zoster virus	Mucoepithelial	Possible oral manifestations of chicken pox and herpes zoster	Chicken pox, herpes zoster
4. Epstein-Barr virus	B-cells and epithelial cells	Hairy leukoplakia, periodontitis, (nasopharyngeal carcinoma)	Mononucleosis, lymphoma
5. Cytomegalovirus	Monocytes, lymphocytes and epithelial cells	Periodontitis?	Mononucleosis
6. Human herpesvirus-6	T-cells and possibly others		Roseola in infants
7. Human herpesvirus-7	T-cells and possibly others		Roseola in infants
8. Human herpesvirus-8	Probably lymphocytes and epithelial cells		Kaposi's sarcoma (in AIDS patients)

the virus earlier in life. EBV's potential for affecting the mouth is further underlined by oral hairy leukoplakia, i.e. white patches typically on the side of the tongue with a hairy appearance, a rare condition restricted to immunosuppressed patients.

Varicella-Zoster virus (VZV) is associated with chicken pox, as a primary infection, and with herpes zoster if reactivated later in life. The vesicular rash formed occurs primarily in the skin, but may also affect the mucosa. The three remaining human herpesviruses (HHV-6, -7 and -8) rarely cause serious disease, but the former two are responsible for a particular type of rash (roseola) with associated fever in infants. HHV-8 is presumably responsible for Kaposi's sarcoma, a rare form of skin cancer seen in immunosuppressed patients.

The herpesviruses typically form chronic infections where the virus remains with its host till death do them apart; much of the time in latency, but with occasional bursts of activity. As these viruses are contact transmitted, either by means of virus production in the skin accompanied by rashes and blister or viral presence in sputum, it is not surprising to find viral activity in the oral cavity. In addition to the traditional clinical picture referred to above, two of them in particular, EBV and CMV have been associated with periodontitis (recently reviewed in Reference (6)). Several laboratories have demonstrated that these viruses are found significantly more frequently in samples taken from affected pockets compared to healthy pockets (7–10), however, this association does not necessarily mean that they are involved in the pathology. Some authors, e.g. Slots et al. (7, 8), suggest that these viruses can influence the development and course of periodontitis, while others are more sceptical, partly due to the low numbers of virus observed (9, 11, 12). Periodontitic lesions may, in part, be the transmission strategy for these viruses, particularly CMV, as replication in the lesions allows

viruses to reach the saliva and thus potentially infecting other individuals (13).

One should be careful when evaluating the presence of low levels of viral genomes in clinical samples as an indication of a viral role in pathogenesis. EBV and CMV are known to be occasionally found in any mouth, as long as a relevant sample of sufficient size is analysed by a sensitive technique. EBV in particular is known to be cyclically active in the body and periodically present in sputum. Thus, the mere presence of viruses in the absence of signs of local viral activity, either in the form of high viral titres or detection of viral RNA or proteins, probably do not constitute any appreciable impact on the aetiology. Moreover, associations such as between viruses and particular bacterial species, or between viruses and the severity of the condition, may be explained by confounding factors; for example, a more active inflammation would be expected to correspond to the presence of particular bacteria, and cause more pain, but also to contain more lymphoid cells and/or more body fluids. The latter factors could explain the association with herpesviruses as both EBV and CMV replicate in leukocytes.

The observation that a patient recovered from a chronic and highly treatment refractory periodontal condition upon antiviral treatment (500 mg valaciclovir, Valtrex®, orally ×2 over 10 days) may be the single most-relevant data suggesting an occasional clinical role for these viruses in periodontitis (14). The initial viral load in this patient was considerably higher than what was observed in any of the other patients tested. Both the clinical condition and the viral load remained stable, and close to the detection level, during a one-year follow-up period after the antiviral treatment. Although the amount of sample obtained from the various teeth examined was not standardised, and is expected to be

smaller when obtained from healthy pockets, the sampling differences can neither explain the three log increase in initial viral load compared to other patients, nor the five to six log drop in viral load observed after treatment. More cases utilising similar antiviral treatments are necessary to provide statistical significance to implicate a potential role of viruses in periodontitis.

Antiviral treatment seems to be the best way to shed light on the actual role of EBV or CMV in periodontitis. Moreover, if successful, it may save the patients from considerable pain and agony. We therefore believe that quantitative antiviral tests, such as real-time PCR, are relevant in the management of these patients, maybe particularly in juvenile and/or chronic and aggressive cases for which other therapy fails. If the tests find appreciable amounts of virus, or other signs of local viral activity, antiviral treatment should be considered as an adjunct to conventional periodontal therapy. It should be noted that the latter form of therapy has also been reported to reduce the viral load (15).

Viral–bacterial interactions

Herpesviruses are well known for their capacity to manipulate the immune system. Although the obvious purpose of manipulation is to boost viral replication, it is easy to envision that a down-regulation of immunological surveillance may also benefit other agents present, such as bacteria. The issue, in regard to periodontitis, has been recently reviewed by Slots (16). Briefly, viral activity in periodontal tissues may impact the local immune response in a way that benefits opportunistic bacteria, and thus leads to aggravated symptoms. For example, the viruses produce cytokine mimics designed to modulate the host's immune defence.

It should be noted that the microbial activity can also induce viral replication, as has been shown recently in the case of EBV and malaria (17). If the impact of viral replication on the bacterial environment is real, then it might be expected that the bacterial profiles would differ between sites with or without virus. Such correlations have been previously reported (16).

Papillomaviruses

Human papillomavirus (HPV) is a DNA virus that can cause chronic infection of either skin or mucosal epithelium. Parts of the viral genome are occasionally integrated in the DNA of the host cells, and some of the genes are assumed to have a malignant potential, as reviewed in Reference (18). The role of HPV in cervical cancer is well accepted and has led to the widespread use of papillomavirus vaccines for young women; related carcinomas occur in the mouth cavity as well as in the oropharyngeal area, however, the role of HPV is less obvious in these cases (19). Based on their putative role in cervical carcinoma, the viruses are classified as having

either high (primarily 16 and 18) or low (primarily 6 and 11) oncogenic potential.

Although the reported prevalence varies considerably, HPVs are common in oral samples such as biopsies or brush samples of mucosa, indeed one laboratory reported that 95% of superficial scrapes from healthy mouths were positive (20). Although HPVs are also found in biopsies from healthy mouths, their prevalence is typically reported to be higher in biopsies from oral lesions such as leukoplakia or cancers. In the former case, the association with oncogenic HPVs is less obvious; while in the case of malignant cancers most laboratories find a definite overrepresentation of the more malignant HPVs (19–22). The observed prevalence of oral cancers are, however, considerably lower than those reported for cervical cancers. Still the case favouring a role of these viruses is reasonably strong.

In future, as those who receive papillomavirus vaccines grow up, it will be interesting to see whether the prevalence of oral carcinomas declines along with the expected decline in cervical cancer. In fact, it has been argued that the vaccines should also be offered to men, partly because they too are at risk for genital cancer, and partly due to the assumed connection with oral cancer (23). The main argument against vaccinating both sexes is that these forms of cancer have a considerably lower prevalence in males compared to cervical cancer in females. As the virus forms chronic infections, vaccination of individuals who already contain the potentially malign subtypes is considered less useful. Nevertheless, it seems reasonable, however, to make the vaccine available at an early age to both sexes.

Enteroviruses

The enteroviruses belong to the family of picornaviruses. Enteroviruses have a single-stranded RNA genome and are classified into five species with all together more than a hundred subtypes. Although the majority of human enterovirus (HEV) infections are asymptomatic, they can cause upper respiratory illness, febrile rash, aseptic meningitis, pleurodynia, encephalitis, acute flaccid paralysis and neonatal sepsis-like disease (24).

As to oral affection, the enteroviruses are primarily associated with hand, foot and mouth disease. This is a febrile illness with tender papulovesicular lesions of both the hand, feet and oral mucosa (25, 26). It occurs mostly in children, but can also affect adults. The association with enteroviruses primarily concerns the type A viruses, e.g. Coxsackie virus A16 and enterovirus-71, but other enteroviruses may also be involved. Herpangina is a related condition where the clinical manifestation is primarily in the oral cavity in the form of ulceration and blisters. Again, the condition is rare and restricted to children.

Detection of viruses in the oral cavity

Viral diagnostics have become more relevant in clinical dentistry. This is partly because of an increased awareness that viruses are possible aetiological agents, and partly because the methods of viral detection have become considerably easier. The preferred methods are based on variants of real-time PCR, which not only offer a test for the viral presence, but also yield quantitative data. The latter point is particularly relevant as several of the viruses in the oral cavity may be prevalent even in healthy mouths. A high viral load in a sample taken from affected tissues may, however, as a rule of thumb, suggest direct involvement in the underlying condition.

One problem is that several viruses that are chronically present in the body can replicate in leukocytes (e.g. EBV and CMV). As the typical clinical samples will stem from inflamed tissues, such as periodontal pockets or ulcerations, one would expect a presence of these viruses; if for no other reason due to the accumulation of leukocytes; a point that has been demonstrated at least in the case of CMV and periodontitis (27). Again, a clinical role is suspected if the titre is particularly high, and even more so if the condition improves upon antiviral treatment.

In order to take samples for detection of viral nucleic acids, whether by PCR or other methods, it is preferable to immediately transfer the sample to a small tube containing lysis buffer. The lysis buffer will block the bacterial activity and stabilise the viral RNA and DNA present. The tubes are advised to be frozen unless the samples are to be tested within a day or two, in which case they may be kept in the refrigerator. Upon arrival in an analytical laboratory, RNA and/or DNA are extracted from the samples, and aliquots added to a reaction mix for PCR.

Standardisation of sampling is a challenge in connection with oral disease. Whether the samples consist of saliva, brush scrapings from mucosa, dental plaque or subgingival plaque, both the actual amount of sample and the content, e.g. contaminants from blood, can vary considerably. Theoretically, one might correlate the presence of virus with other markers in the sample, such as bacterial 16S rRNA or human genes, but that does not offer a convincing standardisation. It seems that the best option is to be careful as to adding relevance to minor amounts of virus, or small differences between samples; however, major differences such as in the antiviral treatment study (14) cannot be due to sampling variations.

A main limitation of PCR-based methods is that they only detect the viruses they are designed to detect. Several novel human viruses have appeared during the last decade, and most likely the human body is the host to a range of viruses that are yet to be described. Moreover, the cost of the methods restricts analyses to a few viral species; thus the total spectrum of potentially relevant

viruses is rarely tested. Two recent strategies compensate for this limitation: microarrays and pyrosequencing. In microarrays, probes detecting different viruses (or other agents) can be applied to a slide and the sample DNA or RNA hybridised onto the slide, thus offering the possible detection of all known viruses. In pyrosequencing, the complete nucleic acids present in the sample are sequenced to look for recognisable viral sequences by searching relevant databases. Both these methods have the same, twofold limitations: one, they are less sensitive than PCR; and two, they are considerably more expensive, although the costs for pyrosequencing is becoming more cost-efficient. Thus, these techniques are not useful for routine diagnostics, but they may be valuable when investigating a possible viral cause of unknown conditions. One such case is the common aphthous ulcers, also known as canker sores. Although various viruses have been implicated by the association (28), it seems unlikely that the true viral culprit, if any, is yet to be found.

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***Bjørn Grinde**

Department of Virology
Division of Infectious Disease Control
Norwegian Institute of Public Health
P.O. Box 4404 Nydalen
NO-0403 Oslo, Norway
Email: bjgr@fhi.no