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REVIEW ARTICLE

Oral transmission of HIV, reality or fiction? An update

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Human immunodeficiency virus (HIV) and many other viruses can be isolated in blood and body fluids, including saliva, and can be transmitted by genital–genital and especially anal–genital sexual activity. The risk of transmission of HIV via oral sexual practices is very low. Unlike other mucosal areas of the body, the oral cavity appears to be an extremely uncommon transmission route for HIV. We present a review of available evidence on the oral–genital transmission of HIV and analyse the factors that act to protect oral tissues from infection, thereby reducing the risk of HIV transmission by oral sex. Among these factors we highlight the levels of HIV RNA in saliva, presence of fewer CD4+ target cells, presence of IgA antibodies in saliva, presence of other infections in the oral cavity and the endogenous salivary antiviral factors lysozyme, defensins, thrombospondin and secretory leucocyte protease inhibitor (SLPI).
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Introduction

Despite the spread of knowledge about safer sexual practices to reduce the transmission of the human immunodeficiency virus (HIV) and the introduction of potent antiretroviral treatments, the pandemic produced by this virus continues to expand at an alarming pace.

Since the beginning of the epidemic more than 20 years ago, over 60 million individuals have been infected by HIV worldwide and more than 20 million of them have died. AIDS is now the fourth cause of death worldwide and the leading cause in sub-Saharan Africa. The WHO estimated that 39.4 million people in the world were suffering from AIDS at the end of 2004 (25.4 million in Africa and 7.1 million in Southeast Asia) and that a third were between 15 and 24 years old. Most sufferers are not aware that they carry the virus. During 2004, 4.9 million new infections were produced (640 000 children) and three million individuals died from AIDS (UNAIDS/WHO, 2004).

Around 610 000 Western Europeans are currently living with HIV/AIDS (only 1.3% of the world total). The prevalence of HIV infection is 0.3% of the adult population in Europe compared with 7.4% in sub-Saharan Africa.

Worldwide, HIV is most commonly transmitted by sexual activity. HIV is found in blood and other body fluids, including semen, vaginal fluid and saliva. The immense majority of HIV infections are produced during unprotected sexual intercourse via the vaginal mucosa and especially via the anal mucosa (Royce *et al*, 1997). The risk of HIV transmission via oral secretions is an issue of growing interest to dental health professionals, above all with the upsurge in the number of infected individuals. Occupational HIV transmission in general has been documented in at least 300 individuals worldwide, mainly nurses (Ippolito, 1989). In dentistry, only three cases have been accepted as occupational transmission, affecting two dentists and a dental assistant (Scully and Porter, 1991), with a further six incidents considered possible cases [Scully and Porter, 1991; Centers for Disease Control and Prevention (CDC), 1993]. The oral transmission of HIV remains a controversial issue and a cause of concern.

The oral–genital transmission of HIV has been suspected by numerous authors (Marmor *et al*, 1986; Perry *et al*, 1989; Mastro and de Vincenzi, 1996). However, epidemiological studies (Rothenberg *et al*, 1998) have reported very little or no transmission by this route. Indeed, many individuals practice unprotected oral sex under the belief that it is safer than vaginal or anal sex, especially members of high-risk groups such as men who have sex with men (MSM) and HIV-serodiscordant heterosexual couples (i.e. one

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partner is HIV+ and the other is HIV−; Gruer *et al*, 1991; Hunt *et al*, 1993; del Romero *et al*, 2002).

Presence of the virus in saliva does not necessarily imply a risk of its transmission to a partner. Thus, hepatitis C virus (HCV) might be present in the saliva of individuals with chronic hepatitis but there is no evidence of its transmission to sexual partners (Fabris *et al*, 1999), and saliva appears to contain only fragments of the virus that are unable to produce the infection alone (Arrieta *et al*, 2001). In the case of HIV, Fultz (1986) reported that saliva can inhibit its replication *in vitro* (Fultz, 1986).

With this background, we sought to address the following questions:

1 Can HIV be transmitted by saliva and/or by unprotected oral–genital or oral–anal contact?
2 Can the HIV in the oral cavity produce infection?
3 What anti-HIV barriers does the oral cavity have? What is the role of salivary anti-HIV factors in this process?

A review of the literature was undertaken for this purpose, compiling data on all factors associated with the presence and infectivity of HIV in genital fluids and saliva, on the HIV-inhibitory properties of saliva and on epidemiological studies of the oral–genital transmission of HIV.

Can HIV be transmitted by saliva and by unprotected oral–genital or oral–anal contacts?

Human immunodeficiency virus is known to be poorly transmitted via oral secretions. Numerous epidemiologic studies have not found HIV transmitted cases related to kissing or sharing kitchen utensils (Friedland *et al*, 1986; Fischl *et al*, 1987; Lusher *et al*, 1991). It is clear that the risk of transmitting HIV via oral sex is markedly lower than the risk of transmission via anal or vaginal sex. However, it is extremely difficult to estimate the precise risk associated with oral exposure because most individuals have various sexual behaviours so that whether the route was oral, vaginal or anal cannot easily be established. Active oral–genital contact (i.e. performance of fellatio) could be expected to carry a higher risk of HIV acquisition compared with passive contact (i.e. receipt of fellatio) because of a series of possible cofactors, such as the presence of oral and/or genital ulcers, gingival bleeding (gingivitis or periodontitis) or the presence of other infections in the oral cavity (Table 1; Scully and Porter, 2000).

Another important aspect is the possible interaction between microorganisms present in the oral cavity and HIV. The presence of human herpesvirus (HHV) is known to modulate HIV-1 replication, and the presence of HHV-8 produced a significant increase in HIV-1 replication *in vitro* and *in vivo* (Mercader *et al*, 2001).

It was recently shown that the presence in the vagina of H_2O_2 - producing microorganisms such as members of the *Lactobacillus* family, also present in the oral cavity, is responsible for sustaining the normal ecological balance at this site. The absence of these microorganisms is related to a higher risk of vaginosis and recurrent infections of the urinary tract by *Escherichia coli*, and to an increased HIV transmissibility (Tomas *et al*, 2004).

Table 1 Factors that increase the risk of HIV acquisition by oral–genital contacts Derived from Scully and Porter (2000)

Traumas
Ulcers or erosions of oral and/or genital mucosa
Gingival inflammation
Sexually transmitted infections (STI)[a]
Ejaculation in mouth (bolus)[b]
Viral RNA[c]
Other oral infections (*Herpes simplex* virus, lactobacillus, etc)

[a]In genital and/or oral cavity.
[b]Receiver of ejaculation.
[c]Measured in genital, anal and salivary secretions.

Some studies have reported that unprotected oral sexual practices carry a greater risk of HIV acquisition, especially in presence of oral ulcers, oropharyngeal inflammation or sexually transmitted infections (STI) in the oropharynx. These STI are more easily transmitted by oral sex than is HIV (Brugha *et al*, 1997). Available data for the UK show that 19% of gonococci cases in male homosexuals and 4% in women are localized in the oropharynx. Moreover, there has been an alarming rise in some STI (e.g. syphilis and gonococci) in most European countries and in the USA, especially among homosexual men, which may be associated with an increase in HIV transmission among these patients (Ashton *et al*, 2003; Giard *et al*, 2003).

As mentioned above, oral sex has always been considered less risky compared with other sexual behaviours, although it does not appear to be definitely risk free. It was reported at the Conference on Retrovirus and Opportunistic Infections in 2000 that eight (7.8%) of 102 recently seroconverted homosexual men had probably become infected by unprotected oral sex (Dillon *et al*, 2000). However, given the large number of active oral sex acts that take place, this suggests that each act carries a low risk. Of the eight individuals infected by this route, three had oral ulcers and seven had made oral contact with infected semen. This possibility was already reported by the CDC in the probable case of an HIV patient infecting an uninfected sexual partner via oral mucosa contaminated with blood of the seropositive patient (CDC, 1997). Cases of HIV transmission via human bite have also been described (Vidmar *et al*, 1996).

One method to determine the risk of orally transmitting HIV is by studying serodiscordant couples who practice unprotected oral sex and are exposed to no other risks for infection. Thus, a 10-year (1989–2000) follow-up study was performed by del Romero *et al* (2002) on 263 stable serodiscordant heterosexual couples whose only risk exposure was oral–genital contact without the use of a condom, with condoms being used for other sexual practices. Despite 10 295 active and 10 658 passive oral–genital contacts, no cases of

seroconversion to HIV were found (del Romero *et al*, 2002). Similar results had been reported in an earlier study by de Vincenzi (1994) of 50 serodiscordant heterosexual couples who practised unprotected oral–genital sex but used a condom for vaginal and/or anal intercourse, with no cases of HIV transmission observed after a 2-year follow-up (de Vincenzi, 1994). Our search of the literature found only one case of oral–anal transmission in which a male homosexual acquired HIV from a seropositive male with gingivitis after unprotected oral–anal sexual activity (Gill *et al*, 1992). Table 2 summarizes the epidemiological evidence on the oral transmission of HIV.

Can the HIV in the oral cavity produce infection?

Human immunodeficiency virus could be present in the genital fluids of infected individuals, in preseminal fluid (Pudney *et al*, 1992), semen and cervicovaginal secretions (Hart *et al*, 1999). Genital ulcers increase the risk of the presence of HIV with infective capacity and the transmission rate is higher in these cases (Dickerson *et al*, 1996).

Human immunodeficiency virus shows tropism for CD4+ receptors in the cell membrane of monocytes and lymphocytes. The most common cell type in the oral cavity is the epithelial cell, i.e. a cell type that does not express the CD4 antigen and is, therefore, not prone to HIV infection (Milman and Sharma, 1994). On the contrary, *in vitro* studies showed that human epithelial cells could be infected by HIV and transfer the infection to adjacent leucocytes, where it can be neutralized by IgA (Lamm, 1997). It has also been demonstrated that the transcytosis of HIV via epithelial cells can be inhibited by S-IgA, IgG or IgM, and that S-IgA is more efficient than IgG (Bomsel *et al*, 1998; Hocini and Bomsel, 1999; Becquart *et al*, 2000). However, *in situ* polymerase chain reaction studies only detected proviral DNA of HIV-1 in 1–4% of epithelial cells taken from saliva (Qureshi *et al*, 1995). It was also reported that other cell types, e.g. uterine cervix or gastrointestinal tract cells, can be infected by HIV (Adachi *et al*, 1987; Liu *et al*, 2003), with the possible participation of galactosylceramide receptors (Yahi *et al*, 1992) and CCR5 and CXCR4 coreceptors (Alkhatib *et al*, 1996; Deng *et al*, 1996; Dragic *et al*, 1996), which have not been described in the epithelium of the oral cavity. The

Table 2 Epidemiological studies on oral transmission of HIV since 1990[a] (adapted from Rothenberg *et al*, 1998)

Study	*Results*	*Main conclusion*
Kuiken *et al* (1990)	Cases: 84 HIV+ MSM Controls: 168 HIV− MSM POI: RR 1.12 (0.59–2.12) AOI: RR 1.50 (0.81–2.78)	Oral exposure was not an independent risk factor
Samuel *et al* (1993)	Cases 83 HIV+ MSM Controls 249 HIV− MSM POI RR 5.3 (2.0–19) AOI: RR 3.6 (1.4–13)	Oral exposure was not an independent risk factor
Raiteri *et al* (1994)	18 serodiscordant lesbian couples practising unprotected oral sex studied for 3 months. No seroconversion	There was no risk of transmission
Ostrow *et al* (1995)	Cases: 76 HIV+ MSM Controls: 389 HIV− MSM POI: RR 0.95 (0.89–1.01)	Oral exposure was not an independent risk factor but there were data at the detection limits
Faruque *et al* (1996)	2323 individuals, 18–19 years POI with oral ulcers: OR 1.9 (1.0–3.6)	Oral exposure was independently associated with HIV, especially among individuals with oral ulcers
Wallace *et al* (1997)	3073 prostitutes POI: more prevalent practice 35.4% less prevalent practice 24.2% ($P < 0.0001$)	Oral exposure was associated with HIV, especially among crack users
Page-Shafer *et al* (1997)	345 HIV+ MSM; 345 HIV− MSM with no POI (per partner) RR 1.05 (1.00–1.11)	Oral exposure was significantly associated with HIV seroconversion
de Vincenzi (1994)	50 serodiscordant heterosexual couples practising protected anal and vaginal sex but unprotected oral sex	After 2-year follow-up no cases of HIV transmission were found
Dillon *et al* (2000)	102 HIV infected MSM 16.4% cases via oral sex	More exhaustive review of cases showed percentage to be 8%
del Romero *et al* (2002)	135 HIV-individuals (110 females, 25 males) performed total of 19000 unprotected oral–genital sex contacts with HIV+ partners during 210/year follow-up	Oral exposure was not an independent risk factor

[a]Only studies performed from 1990 to date are included. Case reports are not included.
MSM, men who have sex with men; POI, passive oral intercourse; AOI, active oral intercourse.

very low presence of HIV-infected epithelial cells in the oral cavity indicates that oral mucosal cells contribute minimally to the viral load in saliva (Shugars and Wahl, 1998).

Recent *in vitro* studies suggested that human oral keratinocytes can be infected by HIV-1 (Moore *et al*, 2003) and that alcohol can favour infection and replication of the virus in lymphocytes. Chen *et al* (2004) reported that alcohol can influence HIV transmission by altering the compartmentalization of CCR4 in epithelial cells of the oral cavity (Chen *et al*, 2004).

Histological studies of tissue samples indicated that epithelial cells are infected by HIV in the basal membrane and migrate to surface layers and then into the oral cavity (Qureshi *et al*, 1997). Mononuclear cells such as lymphocytes, macrophages and Langerhans cells commonly express CD4 surface receptor and HIV coreceptors, also facilitating HIV infection in the oral mucosa (Miller *et al*, 1993; Soto-Ramirez *et al*, 1996). Immunofluorescence and *in situ* hybridization studies confirmed the presence of the virus in mononuclear cells of the gingival crevicular fluid (Suzuki *et al*, 1996) and salivary glands of seropositive patients (Wahl *et al*, 1997). The number of lymphocytes and macrophages markedly increases in the presence of oral infections such as periodontal disease (Offenbacher, 1996), even in patients with intense systemic lymphocyte depletion (Odden *et al*, 1995). Therefore, infection can also result from penetration of the virus through healthy oral mucosa or be secondary to erosions (Hussain and Lehner, 1995).

Although HIV is present in salivary glands (Lecatsas *et al*, 1985), saliva (Groopman *et al*, 1984; Yeung *et al*, 1993) and oral mucosal cells (Qureshi *et al*, 1997), its frequency and infective capacity at these sites are controversial and appear to be clearly lower than in other secretions (Baron *et al*, 1999). In saliva, the frequency of HIV antigen detection has ranged from zero to 35% (Moore *et al*, 1993; Yeung *et al*, 1993; Chebbi *et al*, 1997) and that of proviral DNA and viral RNA detection from 12 to 100% (Yeung *et al*, 1993; Qureshi *et al*, 1995; Liuzzi *et al*, 1996; Chebbi *et al*, 1997), and viral cultures from saliva have shown HIV titres in only 0–39% of cases (Groopman *et al*, 1984; Ho *et al*, 1985; O'Shea *et al*, 1990; Barr *et al*, 1992; Moore *et al*, 1993; Yeung *et al*, 1993; Bergey *et al*, 1994). These findings indicate that saliva may have an inhibitory role (Shugars and Wahl, 1998). Other authors detected intermediate frequencies of HIV in saliva (Kawashima *et al*, 1991; Barr *et al*, 1992; Chebbi *et al*, 1997) with lower viral loads than in plasma (Liuzzi *et al*, 1996). Thus, the saliva of a large proportion of HIV patients does not contain the virus. Theoretically at least, oral traumas, ulcerations and some inflammatory processes such as gingivitis or periodontitis may increase the presence of HIV in saliva (Scully and Porter, 2000).

Jotwani *et al* (2004) reported that healthy gingiva is infiltrated by cells that express all HIV-1 receptors. However, there are very few CCR5+ cells and no CXCR4+ cells in the lamina propria. The number of HIV receptors and CXCR4 and CCR5 coreceptors increases in chronic periodontitis accompanied by a 10-fold increase in alpha-defensin mRNA. T cells, macrophages and dermal dendritic cells were found to be CCR5+ (Jotwani *et al*, 2004).

Shugars and Wahl (1998) detected measurable viral RNA levels in 56% of whole saliva samples and 100% of plasma samples from seropositive patients, with five patients showing HIV-1 RNA levels in saliva up to 62-fold higher than those in blood. Filtration of the saliva significantly reduced the HIV-1 load, indicating that the maximum viral load in oral secretions is predominantly associated with the presence of infected cells or with large cell aggregates that contain the virus. These high concentrations of viral RNA in saliva strongly suggest that cells already carrying the virus penetrate the oral cavity and/or that their active replication takes place in the oral cavity or upper gastrointestinal tract (Shugars and Wahl, 1998).

Oral aphthae can predispose cocaine users to HIV transmission (Faruque *et al*, 1996), and p24+ macrophages have been detected in crevicular fluid, although it is difficult to isolate infective viral particles in this fluid (Chebbi *et al*, 1997). Periodontal disease was not related to greater presence and/or infectivity of HIV in whole blood samples of seropositive patients (Barr *et al*, 1992).

All of the above suggests that saliva may be inhibitory.

What anti-HIV barriers does the oral cavity have? What is the role of salivary anti-HIV factors in this process?

A combination of features of the oral cavity makes it relatively resistant to HIV transmission; a thick epithelial layer, low number of CD4+ target cells and presence of antiviral antibodies and various endogenous inhibitors. Resistance to HIV infection at mucosal surfaces may be also related to HIV-specific CD8+ T cells responses in some individuals and may be the basis for protective vaccine design (Challacombe and Sweet, 2002). Nevertheless, these antiviral mechanisms are not always sufficient, especially if large amounts of HIV enter the oral cavity (as can occur with ejaculation into the mouth) or if continuity of the oral mucosa is lost (as in tearing, ulcers, oral lesions, periodontitis or gingivitis).

A healthy and intact mucosa forms an excellent barrier against infection by pathogenic microorganisms, including viruses (Miller and Cottone, 1993). As well as the lubricating action of the mucosal surface, saliva dilutes the microbial load and expels microorganisms towards the gastrointestinal tract for their inactivation and destruction. However, disruption of the continuity of this barrier caused by trauma or disease may favour penetration of the virus and its replication within susceptible cells, as reported by the CDC in the case of probable HIV transmission via exposure to an oral mucosa contaminated with the blood of a seropositive patient (CDC, 1997). There have also been reports of HIV transmission via bloodstained saliva after a bite

Table 3 HIV-inhibitory activity of different body fluids (Shugars *et al*, 2002)

Mucosal fluid	*Percentage HIV inhibition*	*Relative inhibitory activity*
Colostrum	78.5	High
Whole saliva	72.5	High
Maternal milk (postcolostrum)	68.9	High
Cervicovaginal secretions	51.2	Moderate
Sublingual and submandibular saliva	38.8	Moderate
Seminal plasma	36.7	Moderate
Parotid saliva	13.6	Low

(Vidmar *et al*, 1996). Nakao *et al* (2003) failed to cause oral transmission with or without trauma in mice reconstituted with human peripheral blood leucocytes. However, they concluded that mice provide a useful small-humanized model to help define the window of opportunity for oral transmission by the HIV virus (Challacombe and Sweet, 2002; Nakao *et al*, 2003).

Numerous classical studies have suggested that saliva may inhibit HIV. Over a decade ago, Fultz (1986) published the first study to demonstrate that the whole (mixed) saliva of humans and chimpanzees protects cells susceptible to HIV infection *in vitro*. This inhibition appears to be relatively specific to herpes viruses, which are highly frequent in the saliva of HIV-infected patients (Malamud and Friedman, 1993). Saliva from the parotid and submandibular glands and whole saliva have a certain inhibitory activity against HIV. This activity may be related to the presence of antibodies against HIV or to non-immunological inhibitory factors such as mucins and soluble factors, described below. The absence of salivary inhibitors in some patients has been correlated with the presence of HIV in saliva (Coppenhaver *et al*, 1994). (Table 3).

Antibodies

Salivary antibodies against HIV were identified at the beginning of the HIV pandemic (Malamud and Friedman, 1993). It appears that HIV infection is associated with decreased salivary IgA levels, although a dichotomy has been reported between IgA concentrations in saliva and serum (Challacombe and Sweet, 2002). Thus, the presence of specific anti-HIV antibodies (immunoglobulins IgA, IgG and IgM) can be readily detected in the saliva of seropositive patients (O'Shea *et al*, 1990; Artenstein *et al*, 1997) but at much lower levels than in blood. One study showed that the saliva- and serum-purified IgA of HIV-1-infected individuals could inhibit interaction between gp120 and CD4 (Vincent *et al*, 2004). Detection of anti-HIV antibodies in the transudate of oral cavity fluids has been used as a highly sensitive and specific option for the clinical diagnosis and epidemiological follow-up of the seropositive population (Malamud, 1997). A highly sensitive and specific method to diagnose HIV from gingival crevicular fluid (Orasure^{TM}, Orasure Technologies, Bethelem, PA, USA) was recently approved by the FDA (Burrage, 2003).

Salivary proteins

However, the saliva of seronegative individuals also has protective properties against HIV infectivity (Nagashunmugam *et al*, 1998), indicating that other non-immunological factors are involved in the HIV inhibitory capacity of saliva, including salivary proteins.

The infection of primary monocytes with HIV-1 is significantly suppressed in the presence of human saliva. Human saliva blocked the infectivity of HIV-1 by inverse transcriptase for 3 weeks after a 1-hour exposure of monocytes to the virus, whereas other human fluids failed to reduce the infectivity of the virus (McNeely *et al*, 1995). Submandibular saliva in particular contains high molecular weight glycoproteins, including not only the mucins but also salivary agglutinin (SAG), which displaces HIV envelope glycoprotein gp120 and prevents binding to the CD4 receptor (Nagashunmugam *et al*, 1998). Mucins are densely glycosylated high-molecular weight proteins that sequester and attach to HIV-1, forming complexes that can be eliminated by the host. Parotid saliva contains no mucins but also produces HIV inhibition (Wahl *et al*, 1997).

Researchers at the Cornell Medical Centre (USA) identified a salivary protein that reduces HIV infectivity, a hyperglycoylated high-molecular-weight glycoprotein called thrombospondin 1 (TSP-1) that adheres to HIV surface protein gp120 and strongly inhibits the ability of the virus to enter peripheral blood mononuclear cells *in vitro*. Removal of TSP-1 reduces the anti-HIV inhibitory effect of saliva (Crombie *et al*, 1998). However, removal of all high-molecular weight proteins from saliva does not completely suppress its inhibitory activity against HIV, suggesting that soluble or smaller molecules are also implicated. There are other soluble factors in saliva with anti-HIV properties (Table 4), such as the C1q component of the complement system, which binds to and sequesters the viral particles in presence of fibronectin (Su and Boackle, 1991; Crombie *et al*, 1998). Fibronectin interacts with HIV envelope glycoproteins and can positively participate in reducing transmission of the virus (Llena-Puy *et al*, 2004).

Defensins are peptides of cyclical structure that markedly reduce the lytic action of human T lymphocytes associated with HIV-1 infection, interfering with the entry of the virus into the lymphocyte (Nakashima *et al*, 1993). Defensins are important mediators of the innate defence of mucosae against microbial infections. Several α-defensins and minidefensins are effective inhibitors of HIV-1 infection *in vitro*, and recent evidence implicates α-defensins in resistance to HIV-1 progression *in vivo* (Cole and Lehrer, 2003). It is also known that HIV-1 induces mRNA expression of human β-defensin-2 and -3 in human oral epithelium and that these defensins but not human β-defensin-1 inhibit HIV replication in immunocompetent cells. This inhibition involves a direct binding to HIV-1 and an additional inverse modulation of CXCR4 expression on the cell surface. Quinones-Mateu *et al* (2003) concluded that inhibition of HIV-1 replication by β-defensins may play an important role in protecting the oral cavity and other mucosal surfaces from the infection (Quinones-Mateu

Table 4 Salivary factors with anti-HIV activity

Factor	*HIV-inhibitory mechanism*	*Author*
Anti-HIV antibodies	Neutralize and inactivate the virus IgA inhibits interaction between gp120 and CD4	Miller *et al* (1993) Vincent *et al* (2004) Challacombe and Sweet (2002)
C1q component of complement	In presence of fibronectin, binds to the virus and produces its sedimentation.	Su and Boackle (1991) Llena-Puy *et al* (2004)
Cystatins	Have general antimicrobial activity; inhibit cysteine proteases	Bergey *et al* (1994) McNeely *et al* (1995)
Defensins (α-β, θ defensins and minidefensins)	Have general antimicrobial activity; Block penetration by the virus	Nakashima *et al* (1993) Quinones-Mateu *et al* (2003) Cole and Lehrer (2003)
Lactoferrin	Binds to iron to inhibit bacterial proliferation and viral replication.	McNeely *et al* (1995) Puddu *et al* (1998)
Lactoperoxidase	Inactivates virus by production of hypothiocyanite	Yamaguchi *et al* (1993)
Lysozyme	Interrupts HIV replication by destroying viral membranes	McNeely *et al* (1995) Lee-Huang *et al* (1999)
Ribonuclease	Blocks the reproduction of the virus by destroying its genetic material (metabolize select RNAs)	McNeely *et al* (1995) Saxena *et al* (1996)
Mucins	Sequester and aggregate viral particles	Bergey *et al* (1993); Bergey *et al* (1994)
Secretory leucocyte protease inhibitor (SLPI)	Interact with a cellular surface molecule to limit viral entry into target cells	McNeely *et al* (1995); McNeely *et al* (1997); Shugars *et al* (1997); Wahl *et al* (1997); Farquhar *et al* (2002); Skott *et al* (2002); Lin *et al* (2004)
Thrombospondin 1 (TSP-1)	Produces aggregation of the virus; during penetration by virus, blocks its interactions with lymphocytes	Crombie *et al* (1998)
Proline-rich proteins (PRPs)	Bind to gp120 of the virus, preventing its penetration of lymphocytes	Robinovitch *et al* (2001)
Salivary agglutinin (SAG)/ Mucin MG2	Bind to and displace gp120 from virions Agglutinate HIV and dissociate viral envelope proteins	Challacombe and Sweet (2002); Shugars *et al* (2002)
Hypotonic effect	Lyses HIV-1 infected mononuclear leucocytes	Baron *et al* (2001); Challacombe and Sweet (2002)

et al, 2003). They showed for the first time that HIV-1 induces expression of β–defensins on human oral epithelial cells and that β-defensins block HIV replication by direct interaction with the virions and by modulation of the CXCR4 coreceptor (Quinones-Mateu *et al*, 2003).

In addition, lactoperoxidase released by neutrophils inactivates HIV-1 by the production of hypothiocyanite (Yamaguchi *et al*, 1993). A further two proteins with inhibitory capacity detected in saliva are SAG and mucin MG2, which bind to and displace gp120 from virions giving rise to defective viral particles that cannot infect host cells (Nagashunmugam *et al*, 1998), with MG2 being the most active protein (Shugars *et al*, 2002).

Table 4 lists the numerous salivary proteins with antiviral and anti-microbial properties. In 1999, American scientists discovered that the urine, tears and saliva of pregnant women contain two proteins with potent anti-HIV inhibitory effects (lysozyme and ribonuclease). Furthermore, both types of protein are present in a pregnancy hormone called human chorionic gonadotropin (HCG) and transmission of the virus is extremely rare during the first term of pregnancy, when HCG levels are especially high (Lee-Huang *et al*, 1999).

In vitro studies have shown that the lysozyme protein found in the tears, saliva and urine of pregnant women and the ribonuclease also found in their urine can completely block HIV replication and reduce HHV-8-induced tumours (e.g. Kaposi's Sarcoma). Hypothetically, these proteins could act jointly to attack HIV, with lysozymes destroying viral membranes while ribonucleases block reproduction of the virus by destroying its genetic material, although concentrations above physiological levels would be required (McNeely *et al*, 1995). Saxena *et al* (1996) found a ribonuclease homologous to RNase A named onconase that inhibited virus replication in chronically HIV-1 infected human cells without killing virally infected cells (Saxena *et al*, 1996). Cystatins, present in saliva, have also demonstrated a certain inhibitory activity against HIV, whereas statherin and amylase have no blocking effects on replication of the virus (Bergey *et al*, 1994).

Lactoferrin, secreted by neutrophils and exocrine glands, is found in saliva, maternal milk, tears, semen and other mucosal secretions. It can inhibit HIV replication, both when iron-saturated and when not, and it can also interfere with the adhesion and entry of HIV to host cells (Puddu *et al*, 1998).

Secretory leucocyte protease inhibitor (SLPI) has been isolated in human parotid secretions (Thompson and Ohlsson, 1986). It is a non-glycosylated protein

secreted by acinar epithelial cells of the submucosal glands and can inhibit HIV replication *in vitro* at physiological concentrations (McNeely *et al*, 1995; Shugars, 1999). This inhibition is physiological and dose-dependent, with a maximum inhibition at 1–10 μg ml^{-1} (>90% inhibition of retrotranscription activity). Although the highest concentrations of SLPI are found in saliva, it is also present in semen, cervicovaginal secretions, maternal milk, tears, synovial liquid and cerebrospinal fluid. It is secreted by epithelial cells that coat some mucosal surfaces (Shugars and Wahl, 1998). The mechanism by which SLPI protein inhibits HIV infection appears to be more related to the host target cell than to a direct effect on the virus. *In vitro* studies have demonstrated that SLPI binds to human CD4+ mononuclear cells, blocking infection by HIV. It acts during early stages of the viral life cycle, probably at the time of its penetration into the host cell and at any rate before inverse transcription occurs. The anti-HIV inhibitory activity of whole saliva is reduced but not completely suppressed by SLPI depletion (McNeely *et al*, 1995; Shugars, 1999). It also appears that the inhibitory function of SLPI in human saliva is HIV-1 specific and varies with virus tropism (Skott *et al*, 2002). The fact that SLPI is produced in acinar cells of salivary glands and never accumulates in the stroma of these glands may explain why HIV has been isolated in glandular tissues but not in oral secretions, where it would be inhibited by this protein (Shugars and Wahl, 1998). Salivary flow rate is decreased and the concentration of SLPI is increased in the presence of HIV infection. SLPI concentration in parotid and submandibular/sublingual saliva is greater with HAART, but no association was found between CD4+ cell counts and SLPI concentration in saliva (Lin *et al*, 2004).

No association was found between SLPI levels in the colostrum or postcolostrum breastmilk of seropositive mothers in the Congo and their transmission of the infection to their children. On the contrary, a second study in South Africa found a significant reduction in mother-child HIV transmission in mothers with high SLPI levels in their cervicovaginal secretions at week 32 of gestation but not at delivery (Pillay *et al*, 2001). In addition, elevated SLPI levels in the saliva at 1 month of gestation were associated with a reduced risk of HIV infection among children exposed to the virus via breastfeeding (Farquhar *et al*, 2002; John and Kreiss, 1996).

Robinovitch *et al* (2001) recently demonstrated that basic proline-rich proteins (PRPs) found in human parotid saliva have a potent anti-HIV-1 activity independent of that attributed to SLIPI and TSP-1. Its action mechanism is based on the binding of these proteins to the gp120 of the virus, preventing entry of the HIV into the host cell (Robinovitch *et al*, 2001).

Other factors

Hypotonic saliva inhibits the production of HIV by infected leucocytes, representing a further factor contributing to the extremely low oral transmission of HIV. Salivary hypotonicity appears to destroy the cell wall of HIV-infected mononuclear leucocytes, preventing them from binding to mucosal epithelial cells and producing infective HIV (Baron *et al*, 1999; Baron *et al*, 2001).

It is possible that the natural preventive mechanisms against the oral transmission of HIV could be extrapolated to the vagina and rectum. Indeed, attempts are underway to evaluate the application of these mechanisms to other mucosae, such as rectal and vaginal moose (Baron *et al*, 2001). However, the successful transmission of HIV via seminal fluid and maternal milk has yet to be elucidated. It may be caused by the isotonicity of these fluids, which would prevent the inactivation of HIV-transmitting leucocytes that occurs in saliva (Baron *et al*, 2000). These authors also speculated that potential antiretroviral drugs produced from these proteins may be better tolerated and have lesser secondary effects compared with existing therapies, given that they are naturally produced by the organism.

Conclusions

The scientific evidence gathered in this review suggests that the risk of HIV transmission by oral–genital sexual practices is substantially lower than that carried by genital–genital or genital–anal practices. Exposure to saliva poses a far lower risk compared with exposure to blood. Active oral–genital contact probably carries a higher risk than passive contact. The presence of aphthae, erosions, ulcers or inflammatory processes with bleeding (gingivitis or periodontitis) in the oral cavity of the seronegative individual may increase the risk of HIV transmission (Table 2).

Anti-HIV inhibitory factors in saliva may make a major contribution to the extremely low or negligible rates of oral transmission of the virus reported by epidemiological studies. Each inhibitory factor has its specific function. According to present knowledge, SLPI is the most important factor with the greatest inhibitory activity at physiological doses, although the combined action of molecules such as lysozyme, defensins, ribonuclease and TSP-1 may also play an important role. All of these factors mainly act by inhibiting binding between the HIV and CD4+ lymphocyte and may have potential therapeutic applications, such as virucides in topical preparations.

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