ORAL AND DENTAL ASPECTS OF HIV DISEASE

by

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A thesis submitted to the University of London in accordance with the requirements for the degree of Doctor of Philosophy

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University of London,
January, 1998
ABSTRACT

This thesis has examined various oral and dental aspects of HIV disease. A non-isotopic single-strand conformation polymorphism procedure was developed, to directly monitor the diversity of Epstein-Barr Virus (EBV) carried in tissues and body fluids. Extensive inter-host diversity was observed, and in Human Immunodeficiency Virus (HIV)-infected hosts, EBV diversity could be demonstrated inter-compartmentally, even at sites that were anatomically proximate. In addition, a shift in tissue distribution of EBV-genotypes in hosts co-infected by HIV, was observed. It is suggested that the tongue, in HIV-infected persons, is hematogenously superinfected by Z\textsubscript{2} variants of EBV, which are reported to preferentially carried by peripheral blood and possess 2 copies of a 29-base pair (bp) repeat in the BZLF-1 gene, and that this may play a role in the eventual appearance of Oral Hairy Leukoplakia (OHL).

In United Kingdom, an increasing number of dental students and practitioners were found to be willing to provide dental care for HIV-infected persons. However, their knowledge about HIV infection when tested, was found to be incomplete. Finally, the prevalence of HIV among dental out-patients in an inner-city Dental Hospital in London was found to be 0.4%, using a serological diagnostic test.
DEDICATIONS

To my father, mother and brother
DECLARATION

"Except for the help listed in the acknowledgements, the contents of this Thesis are entirely my own work. This work has not previously been submitted, in part or in full, for a degree or diploma of this or any other University or examination board"

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ACKNOWLEDGEMENTS

I am deeply indebted to Dr Chong Gee Teo, Consultant Virologist, Virus Reference Division, Public Health Laboratory Service, Colindale, for the impeccable supervision of this project.

I am grateful to Drs S. Hinrichsen and J. C. Leao, Universidade Federal De Pernambuco, Recife, Brazil; L.D. Alberti, Department of Oral Medicine and Pathology, Dental School, University of Chiety, Italy and Mrs A.W. Boulter, Department of Primary Dental Care, King’s College, School of Medicine and Dentistry, London, UK, for the provision of study specimens.

I am fully indebted to Professor Stephen Porter for the overall supervision of my thesis, and his invaluable help to complete it on time.

Many thanks to all the members of the Virus Reference Division, Central Public Health Laboratory, Colindale, and Department of Oral Medicine, Eastman Dental Institute for Oral Health Care Sciences for their assistance during my studies.

I am fully indebted to Professor Crispian Scully and his family, for their warm and unaffected hospitality.

I am deeply indebted to Professors Stavros Papanicolaou and George Laskaris for their invaluable help to pursue my studies abroad.

I wish to thank the State Scholarships Foundation, Republic of Greece and the U.K Medical Research Council, for the financial support of my thesis.

Last, but not least, I want to express my deepest gratitude to my family, whose love and devotion are my constant supplies for any productive and constructive work.
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CHAPTER 1

GENERAL ASPECTS OF HIV DISEASE
Introduction

The first detailed report of Acquired Immune Deficiency Syndrome (AIDS), appeared in June 1981 in the *Morbidity Mortality Weekly Report*. Five previously healthy, young, homosexual men, had been treated for biopsy-confirmed *Pneumocystis carinii* pneumonia at three different hospitals in Los Angeles between October 1980 and May 1981. A few weeks later, the same journal carried a further report from the Centers for Disease Control (CDC), of an increasing number of Kaposi’s sarcoma in young homosexual men in New York City and California. In July 1982, the first cases of AIDS in hemophiliacs were reported and, in the same year, the first case of AIDS associated with blood transfusion was recognized. Acquisition of AIDS via heterosexual intercourse was implicated early in 1983.

In May 1983, a new T-cell lymphotropic retrovirus associated with AIDS-like disease, was isolated by French investigators from a patient with lymphadenopathy, and was termed lymphadenopathy-associated virus (LAV) (Barre-Sinoussi et al., 1983). Some months later, American investigators isolated a virus from patients with AIDS, which they named human T-cell lymphotropic virus type III (HTLV-III) (Gallo et al., 1984). Subsequent studies showed that LAV and HTLV-III were the same virus. A third isolate called AIDS-associated retrovirus (ARV) was later found (Levy et al., 1984). In May 1986, an international subcommittee proposed the virus, likely to be the cause of AIDS, to be officially designated as the Human Immunodeficiency Virus, and to be known
in abbreviated form as HIV (Coffin et al, 1986). Subsequently, a further virus was described, termed HIV-2 (Clavel et al, 1986). HIV-2 originated in West Africa and has spread to Central Africa, Europe, USA, South America and United Kingdom (UK), though at present, there are few infected persons reported in developed countries. They have similar biological properties, the same way of transmission, and are equally capable of causing disease. HIV-1/ HIV-2 co-infection can occasionally arise, although HIV-2 infection may have a slower time course to AIDS than HIV-1 (Levy, 1993).

Molecular Biology of HIV

HIV is a retrovirus consisting of an RNA core, surrounded by the p24 core protein and the p17 just inside the viral envelope. The viral envelope consists of the gp41 transmembrane component and the gp120 external glycoprotein. The genome of HIV reflects these structural components and contains three major genes (gag, pol and env). Of these, gag encodes the core protein, pol encodes various enzymes, including reverse transcriptase, and env encodes the envelope glycoproteins. The gene products of pol direct the synthesis of a cDNA copy of the viral RNA, thus reversing the usual method of reduplicating genes. Other parts of the HIV genome are regulatory in function. Notable among these are tat (transactivating region), which is capable of causing marked, even explosive replication of HIV, and nef, which downregulates or inhibits the replication of HIV. Thus, tat, nef and other regulatory genes appear to provide means of controlling the latency and replication of HIV. HIV-1 can be grouped in two
main categories: M, which contains 10 genetically distinct subtypes (A-J), and O, which contains several heterogeneous viruses, while HIV-2 contains at least five subtypes (Scully, 1997).

**Epidemiology**

Nearly 30 million HIV infections and over 8 million AIDS cases had occurred worldwide in adults and children by the end of 1996, according to estimates made by the United Nations Programme on AIDS (UNAIDS) and the World Health Organization (Communicable Disease Report, Public Health Laboratory Service, 1997). The UNAIDS and WHO also estimated that over 6 million adults and children had died from HIV infections by the end of 1996. Many die from HIV disease and opportunistic infections without necessarily developing a condition that satisfies the AIDS case definition. New HIV infections are arising at an estimated rate of 3 million a year, over 95% of them are in the developing world, and the majority of newly infected adults are under 25 years of age (Communicable Disease Report, Public Health Laboratory Service, 1997).

It is estimated, by the year 2000, there will be a worldwide prevalence of HIV infections of 40,000,000, of which 90% will be in developing countries, especially Africa and Asia (Communicable Disease Report, Public Health Laboratory Service, 1997).
The features of the pandemic differ from country to country. Variations in prevalence reflect the timing of the emergence of HIV infection in the population, the rates of growth of the epidemic, and the sizes and types of the patient groups at risk.

A total of 15,074 cases of AIDS have been reported in the United Kingdom between 1982 (when reporting began) and the end of 1997, 73% of whom are known or presumed to have died (Communicable Disease Report, Public Health Laboratory Service, 1997). Like other geographic areas, the number of deaths among those with AIDS in the UK are likely to be underestimated, as many are never reported or are reported too late to be incorporated in timely surveillance data.

It is estimated that over 2,000 new cases of AIDS will arise in the UK each year from 1997 to 1999. Over 8,500 people with AIDS or other forms of severe HIV disease are expected to be alive and requiring care in the UK at the end of 1999. The number of reported AIDS cases and HIV infections per head of population, is five and four times greater, respectively, in the south east of England than elsewhere in the UK (Communicable Disease Report, Public Health Laboratory Service, 1997).

In the UK, sexual intercourse between men is the major route of infection and over 70% of AIDS cases and 60% of HIV infection reports are in homosexual/bisexual men. By the end of 1996, almost 10,000 AIDS cases and
17,000 HIV diagnoses attributed to this exposure had been recorded in the UK. The prevalence of HIV infection among homosexual men attending genitourinary medicine clinics was 9.9% in London and the south east, and 2.5% in the rest of England and Wales. Surveillance of unlinked anonymous blood specimens suggests that a third of these are unaware of their infections (Communicable Disease Report, Public Health Laboratory Service, 1997).

The proportion of reports of HIV infection attributed to heterosexual exposure has risen substantially over the past nine years, from 4% in 1986 to 27% in 1996. This rise in reports of heterosexually acquired infections is due primarily to exposure abroad (74% of heterosexually acquired infections). Sixty-nine per cent (69%) of patients with AIDS and 67% of those with HIV infection associated with heterosexual transmission in the UK were reported from the London regions. In Scotland, 38% of all heterosexual AIDS cases were attributed to exposure to a high risk partner and most (73%) of this group acquired their infection from a partner who had injected drugs (Communicable Disease Report, Public Health Laboratory Service, 1997).

By the end of December 1996, 4% of AIDS case reports and 7% of HIV infections in England, Wales, and Northern Ireland were attributed to infection through injecting drug use; about a third of these cases were women. In Scotland, 35% of AIDS cases and 45% of HIV infections were attributed to injecting drug use and 29% of these were women (Communicable Disease Report, Public Health Laboratory Service, 1997).
In the UK, over 1,200 males and 11 females have been identified as HIV-infected through treatment with blood products, over 600 of whom have developed AIDS. Most patients who receive blood products are males who require clotting factors to treat congenital haemophilia. As the incidence of haemophilia is similar throughout the UK, infections attributed to its treatment have been spread more evenly across the country than cases in other exposure categories. Heat treatment for blood products and donation/donor screening has virtually eliminated new infections through blood products, transfusion, and transplant. There has been little spread to the sexual partners of those infected through blood products, transfusion, or transplantation (Communicable Disease Report, Public Health Laboratory Service, 1997).

Mother to child (vertical) transmission is responsible for a small but increasing proportion of AIDS cases (2%) and HIV infection reports in south east England. Combined with cases whose route of infection with HIV has not been established, they account for only 1% of AIDS cases and 3% of HIV infection reports (Communicable Disease Report, Public Health Laboratory Service, 1997).

**Transmission - Pathogenesis**

Human Immunodeficiency viruses are transmitted by unprotected sexual contact with an infected person, by sharing needles used for injecting drugs, from infected mothers to their infants and through transfusion of infected blood or
blood products. Saliva is not associated with HIV transmission, except when infected blood is present in saliva. More than 90% of the people who are exposed to HIV through the transfusion of HIV-infected blood become infected, making it clear that blood to blood contact is an efficient way to transmit HIV (Chenoweth and Gobetti, 1997).

Human Immunodeficiency Virus binds to and damages host cells bearing the CD4+ receptor, which are mainly helper-inducer T lymphocytes, monocytes and macrophages. Non-CD4+ cell types such as Langerhans cells, astroglial, oligodendroglial, neuronal and colonic cells can also become infected (Weiss, 1993). Binding of HIV to CD4+ target cells involves interaction of the external envelope glycoprotein molecule gp120 with the CD4+ molecule. The virus next enters the target cell, or is internalized through fusion of the viral envelope with the target cell membrane. Next, the viral RNA is subject to reverse transcription. This involves the production of a single-strand DNA copy of the viral RNA and the destruction of the viral RNA. A second strand of DNA is then synthesized. The linear double-stranded DNA becomes circular and then integrates into host chromosomal DNA (pro-virus), which can remain latent for the lifetime of the host cell.

Upon activation, viral genomic RNA is synthesized and transported into the cytoplasm. By action of messenger RNA (mRNA) and other factors, viral proteins are synthesized, processed and assembled. The formation of the full virion involves budding of the virion core through the cellular membrane,
whereby HIV acquires a coat containing envelope glycoproteins to become extracellular mature HIV virion. The processes of viral latency and replication are controlled in part by the HIV regulatory genes, including tat and nef, acting directly or through the actions of host cell factors.

The failure to recover virus from every HIV-seropositive patient, even those who had evidence of CD4+ cell destruction (Andrews et al., 1987), and the identification of viral genes in only one in every 10,000 peripheral blood mononuclear cells (PBMCs), which percentage cannot explain the massive destruction of these cells observed over the course of disease, has led researchers to postulate a number of indirect mechanisms of CD4+ cell destruction as a cause of AIDS.

Several investigators believe that, the process of normal cell death (apoptosis) is accelerated in HIV infection, and is at least partially responsible for the destruction of CD4+ T-cells (Ameisen, 1992; Meynard et al., 1992; Finkel and Banda, 1994). Some superantigens act by binding to and cross-linking common determinants on MHC class II molecules with specific Vβ determinants on the T-cell receptor (TCR), thereby leading to activation, anergy and depletion of class-II-specific (CD4+ cells) expressing certain Vβ determinants. It has been postulated, that HIV may contain a superantigen, which depletes CD4+ cells expressing specific Vβ determinants (Imberti et al., 1991), although others have failed to identify Vβ perturbations in infected individuals (Posnett et al., 1993).
In addition, amino acid sequence similarities between HIV protein and MHC class II molecules suggest that, HIV infection may lead to the generation of antibodies that react with self antigens, resulting in loss of class II-expressing cells (Dalgleish, 1993). Similarly, gp120 can be taken up and processed by CD4+ cells, rendering them susceptible to lysis by cytotoxic T lymphocytes (Germain, 1988). These mechanisms are possible, but their overall contribution to the destruction of CD4+ cells remains to be determined.

Early efforts to quantify HIV in infected patients were hampered by difficulties in culturing the virus and the relatively insensitive detection methods, leading to the conclusion, that the aforementioned indirect mechanisms of CD4+ cell destruction may be involved in HIV pathogenesis.

Improvement in the sensitivity of viral detection methods and better culture techniques began to yield estimates of far greater viral load (Coombs et al, 1989; Ho et al, 1989; Piatak et al, 1993; Cao et al, 1995). The coupling of in-situ hybridization with polymerase chain reaction (PCR) technology has shown a high number of infected cells in the peripheral blood (Patterson et al, 1993). In addition, lymphoid tissue has shown to be a large, previously underestimated, reservoir of HIV (Pantaleo et al, 1993), and there is a direct correlation between the amount of HIV, the in vitro cytopathic effect of the HIV and the rate of CD4+ cell depletion in infected patients (Connor et al, 1993).
Studies indicate that there is probably an adequate level of active infection of CD4+ cells at all stages of disease to account for the loss of CD4+ cells on the basis of a direct viral cytolytic mechanism alone. Two recent studies (Ho et al., 1995; Wei et al., 1995) provided a vivid demonstration that, there is an extraordinarily large amount of HIV being produced during human infection, and that the immune system is highly stressed in its effort to replenish the CD4+ cells that are constantly being lost. The magnitude of the CD4+ T-cell regeneration in response to the HIV infection may be partially responsible both for the increased levels of activation markers on these cells, and the increased levels of apoptosis that have been observed by previous investigators (Ho et al., 1995).

Other studies have recognized potential contributing factors in the immunopathology of AIDS. One such study has determined that, lymph nodes of HIV-infected patients are the sites of massive covert replication of HIV, that is not apparent in the peripheral blood (Embretson et al., 1993). It has been suggested that follicular dendritic cells are the major contributors to viral trapping and clearance (Pantaleo et al., 1994). In contrast, another study has shown that dendritic cells may be responsible for transmitting HIV to CD4+ T-cells, and simultaneously stimulating these cells to produce a rapidly cytopathic infection (Pope et al., 1994).

Other studies suggest that, cytokine dysregulation (such as TNF-α, TNF-β, GMCSF, IFN-γ), further contributes to HIV pathogenesis (Poli et al., 1993; Folks et al., 1989; Fauci, 1993; Pantaleo et al., 1993).
Natural history

Destruction of the T-lymphocytes by various mechanisms produces a profound immunological defect, mainly in cell mediated immune reactivity. This predisposes to viral, fungal and mycobacterial infections. The reduction in CD4+ T-lymphocytes eventually leads to lymphopenia and a fall in the ratio of CD4+: CD8+ T-lymphocytes.

The time for the onset of symptoms varies and may be influenced by source of HIV infection, patient age, gender, drug habits, immunogenetics and other factors (Learmont et al, 1992). Between 30% and 60% of people infected with HIV, experience a syndrome resembling infectious mononucleosis at the time of initial infection. This illness, termed acute retroviral (or seroconversion) syndrome includes fever, myalgia, a maculopapular rash, lymphadenopathy; less commonly, oral ulcers, neurological manifestations, and occasionally, markers of immunosuppression such as, oral candidosis accompanied by leucopenia and an acute reduction in CD4+ lymphocytes (Rabeneck et al, 1990; Dull et al, 1991).

This stage is also associated with a phase of replication of a very homogeneous virus population (Clark et al, 1991; Daar et al, 1991; Zhu et al, 1993). With the onset of the immune response there is a decrease of virus replication (Daar et al, 1991; Koup et al, 1994). During the ensuing stage of clinical latency, there is evidence of ongoing virus replication and vigorous humoral and cellular immune responses (Goudsmit, 1992). The virus populations within the patient are continually evolving during this period (Delwart et al, 1994), presumably in
response to immune pressure, even during the period when the patient is asymptomatic.

Clinical disease is clearly most likely to appear when the CD4+ T-cell counts fall to low levels, when the infected person has reduced defences for other reasons (such as malnutrition), and where there is exposure to potential pathogens. The average time to development of AIDS is about 10-11 years in most adults in the developed world, but about 20% develop AIDS within 5 years, and a very few (about 2%) appear to not develop AIDS over periods as long as 15 years, mostly because they retain active cytotoxic T cells (Feinberg, 1996).

The new definition of AIDS, proposed by the Center for Disease Control (CDC), includes all patients with CD4+ T-cell counts of less than 200 per microlitre (Table 1:1 and 1:2). Apart from the CD4+ T-cell count, % CD4+ T-cell count, % CD8+ T-cell, and CD4+T:CD8+T-cell estimates, other measures of disease progression include markers of immune activation (eg. Beta-2 microglobulin, neopterin), immune dysfunction (cutaneous anergy), virological markers of immune dysfunction (eg. syncytium-inducing HIV-phenotypes, plasma HIV RNA load) and clinical evidence of disease progression (eg. symptoms of AIDS-Related-Complex (ARC), first AIDS defining symptoms, incidence of recurrent AIDS events, tuberculosis, weight loss) (Fahey et al, 1990; Jacobson et al, 1991; Lafeuillade et al, 1994; Tsoukas and Bernard, 1994).
Multiple studies have reported that, increased viral load in the blood correlates with disease progression (Schnittman et al, 1990; Connor et al, 1993). The same findings have been reported in tissues, in particular, the lymph nodes (Pantaleo et al, 1993; Embretson et al, 1993). Viral burden may reflect the replicative and cytopathic properties of the virus. Mellors and co-workers (1995), showed that, a plasma level of 10,000 copies or more of HIV RNA within one year of seroconversion was significantly associated with the risk of progression to AIDS, and that a level of 100,000 copies or more increased the risk of AIDS 11-fold. They also found that patients in the lowest degree of viral load had an 8% probability of developing AIDS within 5 years, whereas those in the highest degree had a 62% probability of developing AIDS within the same time period (Mellors et al, 1996).

Information emerging from studies in acute seroconvertors indicates that, HIV-1 present in a newly infected individual is relatively homogeneous in sequence, even though the corresponding donor harbours a mixture of genotypes. This may imply that, there may be viral selection during transmission, a notion further supported by observations that the transmitted virus is often a minor variant in the corresponding donor (Zhu et al, 1993). The mechanism by which selection occurs during transmission is unknown.

HIV isolated early in the course of infection frequently replicates slowly (Fenyo et al, 1988) and is non-syncytium-inducing (NSI) in vitro (Tersmette et al, 1989a; 1989b). HIV isolated from AIDS patients often replicates more rapidly and in
about 50% of cases, is associated with the emergence of a syncytium-inducing (SI) phenotype (Tersmette et al, 1989a ; 1989b). Although the phenotypic switch from NSI to SI is a useful prognostic indicator, the biological significance of this change *in vivo* is unclear.

**Clinical Features**

The common manifestations of HIV are mainly infections (particularly fungal, viral, mycobacterial), neoplasms (especially Kaposi's sarcoma and lymphomas) and auto-immune disorders. The clinical manifestations of HIV infection are summarized in Table 1:3.

**Laboratory diagnosis**

Apart from the history and clinical criteria, laboratory investigations are indicated after appropriate professional counselling. There may be a lymphopenia, reduced CD4+ T-cell counts in the blood and a reduced CD4+:CD8+ ratio, but these are not specific findings and HIV infection must be confirmed carefully by testing for HIV.

The Enzyme Linked Immunosorbent Assay (ELISA) or agglutination screening tests for serum antibodies are the first step in serodiagnosis. Serum antibodies are usually detectable from about 6-8 weeks after infection, and most persist for life. The ELISA test has a sensitivity of about 98% and a specificity of about 99%
under optimal conditions. It is rapid and easy to use, but there may be false positive or negative results and therefore a positive ELISA result must be re-tested in duplicate samples and, if two of these three ELISA results are positive, a confirmatory test such as Western blotting must be undertaken.

False positives in both ELISA and Western blotting are seen only in about one in 100,000 tests. Indirect immunofluorescence assay (IFA) can allow detection of HIV-specific IgM antibodies, as soon as 5 days after the onset of acute illness, but while, it is as sensitive and specific as Western blot, IFA does not permit precise identification of specific patterns of antibody reactivity.

HIV antigens can be detected in the blood earlier than can antibodies, and assays for the p24 and other antigens are now available. However, antigen assays do not offer great benefits in the initial diagnosis of HIV infection and even antigen assays are occasionally negative in HIV-infected persons (Scully, 1997).

Techniques are also available to detect HIV nucleic acids and can be used to clarify indeterminate Western blot results. For example, the polymerase chain reaction (PCR) is almost certainly very sensitive and able to detect HIV in HIV-infected but seronegative persons. HIV can also be detected by viral culture or testing for HIV reverse transcriptase, but these are not simple tests and lack the sensitivity and reproducibility needed for clinical work. However, they can reveal HIV-1/2 dual infection.
It is important for the diagnosis to apply at least two methodologically different assays for HIV infection and repeat the test 2 to 3 months later (Scully, 1997). Currently, the United States Food and Drug Administration is considering licencing the first number of test systems for HIV diagnosis at home, based on self finger-pricking or saliva collection (Mortimer, 1996).

Management

The management of HIV disease is aimed at delaying the progression of immunodeficiency, preventing the development of opportunistic infections and recognizing and treating the various infections and neoplasms.

The most promising approach to the management of HIV infection was thought to have been inhibition of the HIV reverse transcriptase, using agents such as zidovudine- a dideoxynucleoside analogue. Zidovudine lacks a hydroxyl group on the 3' position in the ribose ring. This analogue of a standard deoxynucleoside is taken up by the cell and phosphorylated to a triphosphate (nucleotide) analogue, the active form of the drug. Once phosphorylated, zidovudine becomes a substrate for DNA synthesis. However, because it lacks a 3' hydroxyl, another nucleotide cannot be added to it because, the 3'-5' phosphodiester bond in the DNA chain is terminated with incorporation of the dideoxynucleoside. Termination of DNA synthesis prevents the virus from replicating and therefore causes viral stasis (Lipsky, 1996).
As the initial drug approved for the treatment of HIV infection, zidovudine has been used to reduce the frequency of opportunistic infections, increase the CD4+ lymphocyte count and slow the progression to AIDS (Volberding et al., 1990; Kinloch-de Loes et al., 1995). The main adverse effect of zidovudine is bone marrow suppression, but nausea, vomiting, headache, fatigue, confusion, malaise and myopathy can also be serious adverse effects.

Didanosine (2,3'-dideoxyinosine, ddi) is another nucleoside analogue inhibitor of HIV with activity similar to that of zidovudine. It can improve cognitive function in HIV-induced cognitive impairment, produce weight gain, reduce p24 antigenaemia and increase CD4+ lymphocyte count (Lambert et al., 1990; Yarchoan et al., 1990). The most frequent adverse effects of ddi are abdominal cramps and diarrhoea due to the osmotic effects of the drug vehicle. Other major treatment-limiting toxicities have included painful peripheral neuropathy, acute pancreatitis and hepatotoxicity (Yarchoan et al., 1990; Lai et al., 1991).

Dideoxycytidine (ddC) (zalcitabine) is another nucleoside analogue inhibitor of HIV reverse transcriptase (Broder and Yarchoan, 1990), available for patients refractory or intolerant to zidovudine. Adverse effects include rash, oral ulceration, fever, peripheral neuropathy and rarely pancreatitis.

With continued use of zidovudine, HIV develops increasing in vitro resistance, and this is clinically associated with more rapid progression to AIDS or death despite therapy with this agent (D'Aquila et al., 1995). Viral resistance to the
nucleoside inhibitors of reverse transcriptase may result from multiple drug resistant strains and can involve at least five different mutations. Some of these mutations may affect the binding site for the phosphorylated drug within the reverse transcriptase (Shirasaka et al, 1995). Resistant strains are seen in more than 90% of patients, who have received zidovudine for more than 18 months (Land et al, 1991). Fortunately, HIV may regain susceptibility to zidovudine after transient discontinuation of the drug (Land et al, 1991), but zidovudine-resistant HIV strains can be transmitted from one person to another (Mohri et al, 1993; Erice et al, 1993).

Lamivudine, a new nucleoside analogue (another one is stavudine), when given in combination with zidovudine, decreases viral RNA and increases CD4+ T-cell counts more than zidovudine alone (Eron et al, 1995). However, when lamivudine alone is used, resistance develops rapidly. Another study showed that, the lamivudine/zidovudine combination led to a resistance mutation that made the virus resistant to lamivudine, but caused the previously zidovudine-resistant virus to become sensitive to zidovudine (Larder et al, 1995).

The lamivudine-selected mutation has also been shown to increase the fidelity of HIV replication, possibly allowing the immune system to become more effective in eliminating the virus (Wainberg et al, 1996). The toxicity profile of the combination in adults is almost the same as with zidovudine alone, and this combination is now often prescribed. Results from meta-analysis suggest that, clinical benefit does occur with this combination (Staszewski et al, 1996).
Reverse transcriptase can be inhibited by agents that are not nucleoside analogues. Nevirapine is the first of a new class of agents (non-nucleoside reverse transcriptase inhibitors) to be licenced. It is more active as a single drug than any nucleoside reverse transcriptase inhibitor, but as with lamivudine, resistant mutants emerge rapidly and plasma HIV RNA values return to near baseline in a matter of weeks or months. Rash is the most frequent adverse event. Preliminary studies with another agent, atevirdine, reported improvement in CD4+ T-cell counts in patients treated with this agent and zidovudine (Reichman et al, 1995). Other non-nucleoside reverse transcriptase inhibitors, include delavirdine and loviride, not yet licenced in Europe.

Another drug class is the acyclic nucleoside phosphonate analogues, which do not require phosphorylation to inhibit reverse transcriptase. One of these, phosphonymethoxypropyladenine, prevented simian immunodeficiency virus infection in macaques, when the drug was administered before exposure to the virus, as well as, immediately after infection (Tsai et al, 1995).

HIV protease inhibitors have proven to be of major significance in HIV therapy. HIV-1 protease is a complex enzyme composed of 2 identical halves or dimers, with an active site located at the base of the cleft created by the dimerization process. Large HIV-1-derived polyproteins are inserted into this cleft, resulting in cleavage to produce proteins essential to virus replication, such as reverse transcriptase, integrase and structural proteins. HIV-1 protease inhibitors inhibit
this reaction thus, preventing the maturation of infectious virions (Kohl et al, 1988).

In December 1995, saquinavir became the first HIV-1 protease inhibitor to receive approval in USA. Although highly active against HIV in vitro, the current formulation has only modest anti-retroviral activity in vivo (as determined by decreases in viral RNA levels). The limited efficacy is likely due to its high first-pass metabolism and subsequent low oral bioavailability. Nevertheless, its activity is enhanced, if higher plasma levels are achieved (Schapiro et al, 1996).

In a recent double-bind study, 302 patients were assigned to 1 of 3 drug regimens: zidovudine plus zalcitabine, zidovudine plus saquinavir or all 3 drugs in combination. After 24 weeks of follow up, the 3-drug combination was more effective than either 2-drug combination in increasing CD4+ T-cell levels (Collier et al, 1996). In another study involving 978 patients, initial data revealed minimal anti-HIV RNA activity with saquinavir monotherapy and only a modest increase in activity when saquinavir was added to zalcitabine; however, 85 AIDS-defining events or deaths were reported in those treated with zalcitabine compared with 46 in those treated with the combination of zalcitabine and saquinavir (Deeks et al, 1997).

Saquinavir appears to be the best tolerated drug, since it has not been associated with any laboratory test abnormalities (Deeks et al, 1997). When added to
zidovudine, saquinavir does not alter or enhance the known adverse effects of either of these drugs (Collier et al, 1996).

In February 1996, ritonavir was approved for use in combination with nucleoside analogues or as monotherapy for the treatment of HIV infection. Ritonavir is a potent *in vitro* inhibitor of HIV-1 protease, but unlike saquinavir, high plasma drug concentrations are achievable with the current oral formulation. Ritonavir has been shown to demonstrate potent and dramatic effects on plasma HIV RNA and CD4+ cell levels (Danner et al, 1995; Markowitz et al, 1995), to delay disease progression and reduce mortality (Deeks et al, 1997). However, ritonavir is the most problematic drug from a safety and tolerability perspective, particularly during the first few weeks of administration. Ritonavir has been associated with mild to moderate diarrhoea, nausea, vomiting, anorexia, headache, fatigue, taste disturbances, circumoral paraesthesia and elevated plasma concentrations of liver transaminases, creatinine kinase and triglycerides (Deeks et al, 1997).

Indinavir was approved for use in the anti-retroviral therapy in March 1996. As yet, there have been no reported data indicating that indinavir delays disease progression or prolongs life. In a study of indinavir versus zidovudine and lamivudine versus combination of indinavir, zidovudine and lamivudine, 24 of 26 patients on the 3-drug combination had undetectable viral RNA levels at week 24. In contrast, 13 of 26 patients treated with indinavir and 0 of 26 patients treated with zidovudine and lamivudine had undetectable levels (Deeks et al,
In addition, patients treated with the combination of indinavir and zidovudine demonstrated a statistically significant decreased risk of developing genotypic resistance to zidovudine and a suggestion of reduced resistance to indinavir, denoting that the combination of the 2 drugs may prove to have more durable activity than either drug used alone (Deeks et al., 1997).

Indinavir is relatively safe and well tolerated. The most important adverse effects have been nephrolithiasis, occurring in approximately 5% of patients over the first year of treatment and indirect hyperbilirubinaemia in 10% (Stein et al., 1996). Other laboratory test abnormalities with indinavir are rare.

Clinical efficacy data for nelfinavir and VX-478 are limited, but nelfinavir appears to be a potent protease inhibitor (Deeks et al., 1997).

Since protease inhibitors have potent inhibitory effects on their own metabolism, specific combinations, taking advantage of pharmacokinetic interactions to increase or prolong drug concentrations and prolong bioavailability may allow dose reductions without sacrificing anti-HIV activity and may also reduce drug toxicities. Data on combination regimens involving saquinavir and ritonavir, although preliminary, are promising (Deeks et al., 1997). Unfortunately, mutations within the protease gene conferring resistance have been described for each of the 4 currently available protease inhibitors (Condra et al., 1995; Eberle et al., 1995; Condra et al., 1996; Molla et al., 1996). Viral resistance is more frequent when HIV is exposed to subtherapeutic levels of a protease inhibitor, thus,
allowing for ongoing HIV replication in the presence of a selective pressure (Deeks et al, 1997). In addition, when protease inhibitors are administered in combination with 1 or 2 nucleoside analogues, the development of mutations conferring resistance is reduced (Condra et al, 1996).

There are many new anti-viral agents for HIV infection under development. Another viral component that seems to be ideal for attack is that of the zinc finger proteins, which do not display mutational changes (Lipsky, 1996). Zinc finger proteins contain zinc, have finger-like loops, and bind to DNA and RNA. Two zinc finger proteins are contained within the nuclear capsid that is necessary for viral packaging and ultimately replication (Lipsky, 1996). Benzamide compounds have been developed that seem to inhibit HIV virus through an interaction with these proteins (Rice et al, 1995). Recently endogenous substances from lymphocytes, chemokines have been isolated (Baier et al, 1995; Cocchi et al, 1995), which may interfere with a newly described co-receptor involved in HIV infection (Balter, 1996). These substances may have therapeutic potential in combating infection with HIV.

Recently, an international panel (Carpender et al, 1996) suggested, that treatment should start when plasma HIV RNA values exceed 5,000-30,000 copies/ml, or when the CD4+ lymphocyte count is less than 500 cells x 10^6/l, or with the onset of symptoms. The use of two drug combinations of nucleoside analogues or didanosine alone is suggested for patients with mild to moderate immunodeficiency, and three drug combinations including a protease inhibitor
are recommended for patients with moderate to severe immunosuppression or high plasma HIV RNA values. A change in treatment should be considered when the plasma HIV RNA value returns to 70% of treatment values, or with a consistent fall in the CD4+ T-cell count, or with the development of new symptoms. A new regimen should include one or more new drugs, which the patient has not previously used and which are not cross-resistant with drugs previously used, in an effort to overcome viral resistance (Carpender et al, 1996).

In addition the British HIV Association, published guidelines on anti-HIV treatment (Lancet, 1997), which summarized as follows:

- treatment should be offered before substantial immunodeficiency ensues.
- initial treatment should include combinations of at least two drugs.
- switches in therapy should involve substitution or addition of at least two new agents.
- viral load and CD4+ lymphocyte measurements are essential.
- reduction in viral load to below the detection level of a sensitive assay represents the optimal treatment response and failure to achieve or sustain this control should prompt consideration of therapy modification. This response is better achieved most reliably with combinations of two nucleoside analogues, plus a third agent (a protease inhibitor, a non-nucleoside reverse-transcriptase inhibitor, or a third nucleoside analogue) or two protease inhibitors.
Aside from anti-retroviral therapy, the management of HIV disease includes psychological and social care, management and prevention of opportunistic infections and prevention of transmission of HIV.

The main problem in the development of an HIV-specific vaccine is that, HIV is within lymphocytes and other cells and thus protected, and that proteins of HIV can undergo changes. Other problems include the lack of suitable animal models, little data on any natural protection against HIV, lack of complete understanding of the pathogenesis of HIV disease, variation in RNA and protein amino acid sequences of HIV between and within patients, and the need for a vaccine that can include both systemic and mucosal protective immunity (Haynes, 1993; Salk et al, 1993). In view of the complex biology of HIV-host interactions, the most fruitful avenue may be the development of multivalent HIV immunogens tailored to HIV isolates in specific geographical locations (Haynes, 1996).

Post-exposure chemoprophylaxis for occupational exposure to HIV in the dental office.

Exposure to blood-borne pathogens, including HIV, poses a serious occupational risk to health care workers, including dental professionals. However, dental professionals have a low occupational risk of being infected with HIV, despite occupational exposure to people with HIV and frequent accidental skin punctures from instruments (Gooch et al, 1995). This is because, HIV is found in very low
concentrations in the saliva of people infected with HIV, which may be a result of anti-HIV activities found in the saliva (Shine et al, 1997). Despite the high frequency of sharp injuries in medical and dental practice, few health care workers become infected with HIV, as a consequence of accidental injury.

By the end of 1997, 95 definite and 191 possible HIV seroconversions after exposure in the course of health care work had been reported worldwide (Communicable Disease Report, Public Health Laboratory Service, 1998). Of them, 4 definite and 8 possible cases occurred in the United Kingdom. Eighty-nine per cent (85/95) of the definite occupationally acquired infections followed percutaneous exposures. Thirty-nine per cent of the overall total were nurses and midwives, 16% doctors or medical students, 13% clinical laboratory workers and 3% dentists or dental workers (Communicable Disease Report, Public Health Laboratory Service, 1997). The average risk of infection for dental health care workers, attributed to needle punctures and similar percutaneous injuries involving HIV, is approximately 0.3% (Gerberding, 1996).

Chemoprophylaxis is recommended to health care workers who experience occupational high-risk exposure to HIV-infected tissue. The highest-risk exposures are deep (intramuscular) injuries with a large hollow needle that previously had been in a patient’s blood vessel, especially in blood containing high-titer HIV viremia, such as, during acute retroviral illness or end-stage AIDS (Chenoweth and Gobetti, 1997).
Chemoprophylaxis should be initiated promptly, preferably within 1 to 2 hours after exposure. Prophylactic therapy should be continued for 4 weeks. Zidovudine should be considered for all post-exposure chemoprophylaxis. Lamivudine should be added to zidovudine therapy to enhance anti-retroviral therapy, while indinavir should be added for the highest-risk exposures or when zidovudine-resistant strains are likely (Chenoweth and Gobetti, 1997). For exposures with negligible risk such as, contact with saliva or blood contact with intact skin, chemoprophylaxis is not recommended.

Health care workers who experience occupational exposure to HIV, should receive follow-up counseling and medical evaluation. The presence of antibodies for HIV should be tested at baseline, 6 weeks, 12 weeks and 6 months after exposure. Of course, one of dental practitioner’s top priorities should be to protect themselves from injury, by following rigorous infection control practices.

**Paediatric HIV infection and AIDS**

By the year 2,000 there will be six million pregnant women and five to ten million children infected with HIV. Since most children, who become infected acquire the virus from their mother, the future impact of paediatric HIV infection will be directly related to the increasing incidence of infection among women (Scarlatti, 1996).
The rate of vertical transmission of HIV has been evaluated in several epidemiological surveys, and varies from 13% to 42%. It seems that, at least 65% of children are infected during the final 6 weeks of pregnancy and at delivery (Bryson et al., 1992; Rouzioux et al., 1993).

*In vivo* infection of placental cells has been shown as early as 8 weeks' gestation (Lewis et al., 1990), and may therefore account for early in-utero transmission, although this is not still clear. Transmission during delivery may be due to mixing of maternal and fetal blood during contractions, to contamination through mucous membranes, or via swallowing infected maternal blood or cervico-vaginal secretions, when the fetus passes through the birth canal. Transmission through breastfeeding seems to be related to the virus load in breast milk, as well as, to the length of time the child was fed (Scarlatti, 1996).

It has been suggested that detection of HIV virus by polymerase chain reaction within 48 hours of age indicates that, infection of the child took place during gestation, whereas delayed appearance of virus implies intra-partum transmission (Bryson et al., 1992). There is a strong correlation between low maternal CD4+ T-lymphocyte counts at delivery and increased risk of transmission, while mothers who were p24 antigen positive had a three-fold increase in the risk of transmission by comparison with antigen-negative mothers (European Collaborative Study, 1992). In addition, differences in virulence or cell tropism of virus strains harboured by the mother may well contribute to the risk of transmission (Kliks et al., 1994; Scarlatti et al., 1993).
Early diagnosis of HIV infection in children born to seropositive mothers has been hampered because, the conventional antibody tests cannot be used owing to the persistence of placentally transferred maternal IgG, which can be detected as long as the age of 18 months. IgA and IgM do not cross the placenta, so detection of HIV-specific antibodies of such classes in a child indicates infection. However, IgA is detected in only half of the infected infants by the age of 3-6 months, and IgG antibodies of maternal origin have to be removed before testing owning to competition with the antigen. Detection of IgA seems to be a more sensitive assay than detection of HIV-specific IgM, probably because IgM production is transient.

Virus culture and polymerase chain reaction (PCR) are the most reliable techniques for determining infection during the first 2 months of age in non breastfed children, attaining a sensitivity of 90% compared with 50-60% for other diagnostic tests. Interestingly, cases in which, children have lost the maternal antibodies, never seroconverted and remained well during follow up, have been documented in a range from 3 to 6% (Roques et al, 1995; Newell et al, 1996).

Patterns of disease expression and progression differ among HIV-infected children. Most children with maternal acquisition of HIV-infection will display features of HIV infection within 6 months of life. Some children do not have any signs or symptoms of disease by the age of 8-10 years (European Collaborative Study, 1994). The mortality rate for children, who develop features of AIDS
early in life is substantially higher than for those who become symptomatic later during childhood (Scott et al., 1989).

Children are more likely than adults to develop *pneumocystis cariini* pneumonia, with a peak incidence between 3 and 6 months of age, and less likely to develop other opportunistic infections such as toxoplasmosis, tuberculosis, cryptococcosis, and histoplasmosis. Serious bacterial infections, cytomegalovirus infection, lymphoid interstitial pneumonitis (LIP) and encephalopathy are more common in infected children than in adults. By contrast, children rarely develop kaposi’s sarcoma or other HIV-associated tumours. Progressive encephalopathy has been reported as the first manifestation of HIV infection in around 10-15% of children and is associated with poor prognosis (Tovo et al., 1992).

Vitamin A, which is more widely available than zidovudine and less costly, shows promise in preventing perinatal transmission of HIV. A recent study of 118 infants born to HIV-infected women in South Africa indicated that, vitamin A supplementation may significantly reduce morbidity for almost every condition related to HIV-infection (Coutsoudis et al., 1995). In addition, because transmission from mother to infant seems to occur predominantly in the latter stages of pregnancy and delivery, cesarean section might prevent transmission of the HIV virus by avoiding fetal contact with contaminated blood and cervical secretions (European Collaborative Study, 1994). Indeed, it has been estimated that cesarean section may cut the rate of HIV transmission in half (Kuhn et al., 1996).
CHAPTER 2

ORAL MANIFESTATIONS OF HIV DISEASE
Introduction

Oral manifestations of HIV disease are often clearly visible and can be diagnosed on clinical features alone. In cases where HIV status is unknown, and where HIV testing is difficult, certain oral lesions like oral hairy leukoplakia and oral candidosis can be helpful as clinical markers in suggesting immunodeficiency and particularly HIV infection. Oral lesions may also predict progression, or can be used as entry or end points to therapy and vaccine trials, in staging and classification systems and finally as determinants of anti-opportunistic infection and HIV therapy (Greenspan, 1997). The HIV-associated oral lesions and their management are summarized in Tables 2:1 and 2:2.

1. INFECTIONS

Fungal Infections

Oral Candidosis

The profound immunodeficiency, particularly affecting the T helper cells, of HIV infection, gives rise to a spectrum of secondary infections of which oral candidosis is the most common. Indeed, the first documented patients with AIDS had oral candidosis (Gottlieb et al, 1981). The frequency of oral candidosis varies between surveys, depending on the patient selection, disease state, diagnostic criteria, sampling methods etc (Schiodt et al, 1990; Feigal et al, 1991; Ficarra et al, 1994a). In general, the prevalence of oral candidosis in HIV-

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infection ranges from 7% to 93% depending on the stage of HIV disease and the study population (Samaranayake, 1992; Ficarra et al, 1994a; Ramirez-Amador et al, 1993; Gillespie and Marino, 1993; Lamster et al, 1994; Ceballos-Salobrena et al, 1996).

Oral candidosis may be the initial manifestation of HIV disease (Samaranayake and Holmstrup, 1989), and it has arisen in association with the acute retroviral illness that characterizes early HIV infection (Dull et al, 1991). The odds of having oral candidosis has been shown to be 6 times greater for subjects with CD4+ lymphocytes counts between 200-400/mm³ and 23 times greater for subjects with CD4+ counts less than 200/mm³, compared to subjects with CD4+ counts of 400/mm³ or greater (Barr et al, 1992a). A positive association between the presence of oral candidosis and severe immunosuppression has been demonstrated in subsequent studies (Kolokotronis et al, 1994a; Glick et al, 1994a). However, Hamilton and co-workers (1992) could not correlate the Walter-Reed classification scheme with the presence of subclinical colonization, suggesting a more complex pathogenesis for HIV-related oral candidosis.

Oral candidosis may also imply concurrent esophageal candidosis (Tavitian et al, 1986a; Tindall et al, 1989; Connolly et al, 1989) and may be a predictor of liability to other opportunistic infections (Imam et al, 1990). In one study (Klein et al, 1984), in 22 previously healthy adults with unexplained oral candidosis, 19 individuals had a reversed CD4+:CD8+ lymphocyte ratio and 20 had generalized lymphadenopathy, when compared with 20 similar patients with a reversed
CD4+/CD8+ ratio and generalized lymphadenopathy who did not have oral candidosis. Thirteen of the 22 with oral candidosis (59%) acquired a major opportunistic infection or Kaposi’s sarcoma after a median interval of 3 months, as compared with none of the 20 patients with generalized lymphadenopathy and immunodeficiency but without candidosis. Katz and co-workers, (1992) reported that 31% of HIV-infected patients with oral candidosis developed AIDS within 6 months compared to 1% of the HIV-infected persons with healthy oral mucosa. Similar findings have been reported in other studies (Romanowski and Weber, 1984; Chandrasekar and Molinari, 1985; Coates et al, 1992; Lifson et al, 1994; Maden et al, 1994; Ramirez-Amador et al, 1996a; Phelan et al, 1997).

The recommendations of the International AIDS Society of USA, for anti-retroviral therapy for HIV (Carpender et al, 1996), include mucosal candidosis among these features which trigger the recommendation for anti-retroviral therapy. Clinical conditions that include oral candidosis were used as endpoints in a trial of zidovudine in HIV-infected people with CD4+ T-cell counts of > 400 cells/mm^3 (Cooper et al, 1993). The Walter Reed classification system (Redfield et al, 1986), and the simplified classification system of Royce (Royce et al, 1991), place great emphasis on oral candidosis, as a clinical marker to define the stages of HIV disease. Subsequently, other studies have suggested that the diagnosis of oral candidosis may be worthy of note in staging patients with HIV disease (Galli et al, 1995; Begg et al, 1996).
Oral candidosis in HIV disease may present as four distinct clinical variants: Thrush (acute pseudomembranous candidosis), erythematous candidosis, angular stomatitis (cheilitis) and hyperplastic candidosis (Samaranayake, 1992), while median rhomboid glossitis and palatal papillary hyperplasia have recently been suggested to be included as distinct variants of HIV-related oral candidosis (Kolokotronis et al, 1994b; Reichart et al, 1994).

The erythematous candidosis is the most frequent type of oral candidal infection in HIV-disease, as approximately 50% of affected patients have these lesions; pseudomembranous candidosis and angular cheilitis are the next most common entities. Hyperplastic candidosis is uncommon (Samaranayake, 1992). A characteristic feature of HIV-related oral candidosis is the presentation of the disease in multiple oral sites (Samaranayake, 1992; Touyz et al, 1996). Symptoms, which do not always coincide with the appearance of infection, may include burning, soreness or bad taste.

The erythematous form of candidosis presents as pink or red macular lesions typically on the palate and dorsum of the tongue. When affected, the dorsum of the tongue becomes depapillated. The palatal or lingual lesions may appear in apposition with each other. It has been suggested that the erythematous appearance is a secondary consequence of shedding of the plaque of pseudomembranous candidosis, however, in HIV-infection, the erythematous lesions precede the pseudomembranous type, although further studies are needed to confirm this (Pindborg and Nielsen, 1989). At least two studies, have showed
that erythematous candidosis, which is readily overlooked during oral examination, is as significant as the more obvious pseudomembranous type, as a predictor of the subsequent development of AIDS (Dodd et al., 1991a; Nielsen et al., 1994).

The acute pseudomembranous type is characterized by the presence of yellow-white, loosely adherent plaques, on a red or normal coloured mucosa, located anywhere in the mouth. Upon scraping, the white plaque can be removed to reveal bleeding surface. The disease is usually acute, but in HIV-infected cases it may, if untreated, persist for several months when the course appears more chronic than acute (Samaranayake, 1992).

The hyperplastic form of HIV-associated candidosis is characterized by white plaques, which cannot be removed by scraping; it is most often seen bilaterally on the buccal mucosa and rarely in the retrocommissural area, which is the typical presentation site in non-HIV-infected persons (Samaranayake, 1992). Angular cheilitis presents as cracking, fissuring, and redness at the commissures; these lesions may be unilateral or bilateral and may be seen in association with any of the intra-oral presentations (Samaranayake, 1992).

Histologically, the epithelium may be invaded by numerous hyphae or pseudohyphae, without the characteristic massive infiltrate of polymorphonuclear leukocytes (Samaranayake and Holmstrup, 1989). Definitive diagnosis (rarely justified), may require positive morphologic verification by staining with
potassium hydroxide [KOH], Periodic Acid-Schiff (PAS), or Gram-stain (Samaranayake and Holmstrup, 1989).

The levels of *Candida albicans* and other yeasts are increased both in saliva and on the oral mucosa, even in the absence of clinical disease (Tylenda et al., 1989; Fetter et al., 1993; Fong et al., 1997). In general, the frequency of isolation of *Candida* species increases with increasing severity of HIV disease (Torssander et al., 1987), and with reducing CD4+ lymphocyte counts and/or CD4+:CD8+ ratio (Fong et al., 1997).

Although, *Candida albicans* is responsible for more than 85% of clinical oral candidosis in HIV-infected persons, the incidence of infection with *non-albicans* species appears to be increasing as *C.krusei*, *C.tropicalis*, *C. parapsilosis*, *C.lambica*, *C. kefyr*, *Torulopsis glabrata*, *C. lipolytica*, *C. guilliermondii*, *C. sake*, *C. paratropicalis*, *C. stellatoidea*, *C. famata*, *C. inconspicua* and *C. dubliniensis* may also be detected (Tylenda et al., 1989; Franker et al., 1990; de Bernardis, 1992; Powderly et al., 1992; Hauman et al., 1993; McCreary et al., 1995; Silverman et al., 1996; Sullivan et al., 1993;1995;1997; Tumbarello et al., 1996; Dronda et al., 1996; Maenza et al., 1997; Moran et al., 1997).

In HIV-disease, there may be early preferential selection of a subpopulation of *C. albicans*, which possesses increased virulence (Ollert et al., 1995; de Bernardis et al., 1996) or an increased ability to adhere to the oral mucosa (Sweet et al., 1995).
Altered levels of salivary antimicrobials, such as calprotectin, may have a variable effect on the susceptibility to candidal infection (Muller et al., 1993).

Eradication of *Candida* from the oral mucosa is unlikely. Treatment regimens focus on control of symptoms rather than on attempts at *Candida* eradication. Early treatment of oral candidosis may be warranted not only because of the associated discomfort, but also because the oral mucosa may act as reservoir of organisms for spread of disease particularly to the esophagus (Gazzard and Smith, 1990). Associated xerostomia, which has been shown to be an independent predictor of HIV-related oral candidosis (McCarthy et al., 1991; McCarthy, 1992), can be treated with conventional measures (Silverman, 1989) and tobacco smoking should be discouraged.

A wide variety of agents are available for the treatment of oral candidosis. Some of these antifungal drugs are used topically, others systemically by the oral or intravenous routes. Topical therapy requires sufficient contact time between the drug and the oral mucosa and the presence of adequate saliva to dissolve the medication in the case of troches, pastilles and tablets. Sipping water while using the topical antifungal drugs may improve efficacy. The use of a topical fluoride rinse should be encouraged, since several topical drugs contain sweetening agents and long term use together with any xerostomia may lead to an increase in caries.

Nystatin and amphotericin B are the polyenes commonly used for the treatment of oral candidosis. Polyene antifungal agents damage cell membranes by binding
to ergosterol and lead to cell death. Nystatin is administered as oral pastilles, oral suspension or as a cream or ointment. Topical therapy should be continued for 14 days and the effectiveness of treatment depends on compliance. However, relapses are common mainly attributable to the underlying immunodeficiency and to poor patient compliance (Scully et al, 1991a). For this reason, azole compounds, mainly the imidazoles-clotrimazole and ketoconazole-and the triazoles-fluconazole and itraconazole-are alternatively used.

Azoles are thought to be fungistatic and act by inhibiting the synthesis of ergosterol, which thereby changes membrane permeability. Oral ketoconazole is normally given in doses of 200-400 mg daily, and it is usually recommended that the drug is taken with food, since gastric acid is essential for its dissolution and absorption. However, ketoconazole therapy is associated with a number of side effects such as nausea, rashes, pruritus, adrenal suppression and more significantly liver damage, which necessitates the regular monitoring of liver function in all patients on ketoconazole for more than few days (Scully et al, 1991a).

Fluconazole and itraconazole have better pharmacokinetic properties than ketoconazole, as they are water soluble, bind to proteins minimally, they are well absorbed by the gut, have long half lives and are principally excreted by the kidney, with mild and infrequent toxicity. Fluconazole, in daily doses of 50-800mg or 50-200mg weekly, has been reported to be adequate for prophylaxis (Dupont and Drouhet, 1988; Chave et al, 1989; Leen et al, 1990; Stevens et al,
1991; Just-Nubling et al, 1991; Marriott et al, 1993), and may induce a more rapid clinical response, than other azoles in the treatment of mucosal candidosis (de Wit et al, 1989; Ansari et al, 1990; Koletar et al, 1990; Barchiesi et al, 1992; Laine et al, 1992; Pons et al, 1993). Itraconazole, administered as 100mg tablets once or twice daily has elicited a clinical response similar to that of fluconazole (Smith et al, 1991a) and better than that of ketoconazole (de Repentigny and Ratelle, 1996), in HIV-infected people.

The widespread use of azoles as therapeutic and prophylactic agents, has been followed by a rapid increase in reports of Candida species and strains with reduced in vitro and in vivo responsiveness to ketoconazole (Gallagher et al, 1992; Korting et al, 1992), fluconazole (Willocks et al, 1991; Heinic et al, 1993a; Johnson et al, 1995; Moran et al, 1997) and clotrimazole (Lucatorto et al, 1991; Le Guennec et al, 1995).

The overall frequency of resistance to azoles has been estimated to range between 0.1 and 10% (Ng and Denning, 1993; Troke and Hitchcock, 1994). The most thorough studied azole-resistant Candida isolates so far, have been uniformly
cross-resistant, although lack of cross resistance has also been documented (Johnson et al., 1995). It is likely, that multiple and diverse factors play a role in perceived or genuine resistance to antifungal agents, both in vivo and in vitro.

Retrospective surveys indicate that, HIV-infected patients with resistant C. albicans isolates have generally received more frequent therapy for oropharyngeal candidosis (Tumbarello et al., 1996; Maenza et al., 1997; Laguna et al., 1997), or are more profoundly immunosuppressed than patients from whom more susceptible isolates are recovered (Revankar et al., 1996; Laguna et al., 1997).

Some studies have shown that fluconazole resistance can arise within a single strain of Candida, which had been susceptible to fluconazole (Redding et al., 1994; Sangeorzan et al., 1994), while other studies have concluded, that the emergence of the resistance to fluconazole may represent a relapse due to the selective overgrowth of isolates with more resistant DNA subtype under the influence of fluconazole therapy (Barchiesi et al., 1995; Lischewski et al., 1995; Johnson et al., 1995; Boerlin et al., 1995a; 1996). Interestingly, Johnson and co-workers (1995) reported that, in one of their patients, the resistant strain was replaced by a susceptible one, within 11 weeks of cessation of fluconazole treatment, suggesting that, the continued presence of drug is required for resistance to be expressed. In addition, genotypically identical isolates of C. albicans may undergo coordinated phenotypic switching, and thereby, become less susceptible to azoles (Gallagher et al., 1992).
Superinfection or transmission between patients, by other strains of oral \textit{C. albicans} may occur (Miyasaki \textit{et al}, 1992; Boerlin \textit{et al}, 1995b), while the demonstration of diversity of DNA types among isolates from the oral cavity of HIV-infected individuals with oral candidosis refutes the notion of a clonal origin for fluconazole-resistant strains among HIV-positive patients (Le Guennec \textit{et al}, 1995; Tsang \textit{et al}, 1995; Reynes \textit{et al}, 1996; Berenguer \textit{et al}, 1996; Diaz-Guerra \textit{et al}, 1997). The possibility of a dual pattern of infection (reinfection and recurrence) has also been suggested (Bart-Delabesse \textit{et al}, 1993; Powderly \textit{et al}, 1993; McCullough and Hume, 1995).

Failure of azole treatment does not necessarily imply development of resistance or acquisition of a resistant isolate. A full drug history should be compiled, as patients are often taking a variety of drugs which, through pharmacodynamic drug interactions, may lower the concentration of the effective antifungal drug. The serum concentration of the drug should be checked, and a subtherapeutic concentration should alert the physician to the possibility of non-compliance or drug interaction. Poor absorption of the active drug may also be a problem in HIV-associated candidosis. Ketoconazole and itraconazole are best absorbed in an acidic environment, but many HIV-infected persons suffer from achlorhydria and may be inadvertently received a decreased dose, even with regimens that use larger than usual doses. Of course, patient compliance should also be checked.
Additionally, local nebulised pentamidine (Nolan et al, 1994) and thymopentin oral inhalations (Coppola et al, 1996) have been shown to be effective in the management of HIV-related oral candidosis. Gentian violet, whose mode of action is not known, and chlorhexidine rinse an effective antibacterial agent may also be helpful in treating oral candidosis (Ferretti et al, 1988; Nyst et al, 1992).

**Other fungal infections**

Orofacial lesions caused by the main systemic mycoses (namely, cryptococcosis, histoplasmosis, aspergillosis, blastomycosis, coccidioidomycosis, zygomycosis, and paracoccidioidomycosis) may occur in isolation, but are typically associated with lesions elsewhere, often in the respiratory tract, and in HIV disease, are usually seen when there is profound immunodeficiency (de Almeida and Scully, 1991).

The route of transmission of these agents is through inhalation or ingestion of spores. Although in healthy individuals, infection in endemic areas is often asymptomatic and may resolve spontaneously, immunocompromised persons are at particular risk from their dissemination (de Almeida and Scully, 1991). Oral lesions in HIV-infected persons, of blastomycosis, coccidioidomycosis, paracoccidioidomycosis and zygomycosis have not yet been reported (Scully et al, 1997).
Cryptococcosis is caused by Cryptococcus neoformans, a ubiquitous yeast found especially in pigeon faeces and may present in soil. Most patients with disseminated cryptococcosis have cryptococcal meningoencephalitis at the time of diagnosis, and if untreated this is fatal in over 70% of cases (Scully et al., 1997). Cryptococcosis is an indicator disease of AIDS, occurring late in the course of HIV infection, when the median survival may be less than 6 months (Scully et al., 1997).

To date, oral Cryptococcus infection has been observed in a few persons infected with HIV, -most of the affected cases have had disseminated disease-, manifesting as, non-healing extraction wounds, or chronic ulceration of the palate or maxillary gingiva or tongue (Glick et al., 1987; Lynch and Naftolin, 1987; Dodson et al., 1989; Lynch, 1990; Tzerbos et al., 1992; Lucatorto and Eversole, 1993; Schmidt-Westhausen et al., 1995; Piluso et al., 1996). In addition, the occurrence of aggregates of C. neoformans within a palatal Kaposi’s sarcoma, (Kuruvilla et al., 1992), in parotid and labial salivary glands (Monteil et al., 1997), and in perioral and labial umbilicated papular lesions (John Hicks et al., 1997), in patients with AIDS, have also been reported.

Histoplasmosis, caused by a soil saprophyte Histoplasma capsulatum, is the most frequently diagnosed systemic mycosis in the USA and in endemic areas (mainly the Ohio and Mississippi valleys), as over 70% of adults are infected, typically subclinically. Spores are found especially in bird and bat faeces. In
HIV-infection, histoplasmosis arises from reactivated latent infections (Scully et al, 1997).

Oral lesions in HIV infection have been recorded mainly in persons with pulmonary or disseminated histoplasmosis; they are usually ulcerative or nodular, located on the tongue, palate, buccal mucosa or gingiva, and rarely have invaded the mandible (Werber, 1988; Fowler et al, 1989a; Huber et al, 1989; Oda et al, 1990; Cohen et al, 1990; Eisig et al, 1991; Jones et al, 1992; Heinic et al, 1992a; Lucatorto and Eversole, 1993; Liang et al, 1993; Swindells et al, 1994; Souza Filho et al, 1995; Cole and Grossman, 1995; Chinn et al, 1995; Piluso et al, 1996; Nittayananta et al, 1997; Warnakulasuriya et al, 1997).

In addition, necrotic ulcers associated with invasive aspergillosis, (Rubin et al, 1990; Napoli and Donegan, 1991), penicilliosis caused by Penicillium marneffei (Vithayasai and Vithayasai, 1993), and erythematous gingiva associated with geotrichosis (Heinic et al, 1992b), have been observed in HIV-infected patients.

Viral Infections

Whereas HIV infection predisposes to opportunistic infections, certain viral opportunists, such as Cytomegalovirus (CMV) and Epstein-Barr (EBV) virus, are capable of facilitating dissemination and expression of HIV, while concomitantly affecting the immune response (Szigeti et al, 1982). Co-infection of cells may augment expression, and viral replication of one, the other, or both viral agents,
and infection by one virus may engender an increased susceptibility to another (Nelson et al., 1990). Most of these viral agents are latent endogenous herpes group viruses. As a consequence of HIV-infection of CD4+ lymphocytes, macrophages or Langerhan’s cells, many levels of the immune response are disturbed, and the normal balance that maintain non-lytic latent infection is impaired, promoting activation. As the opportunists become pathogenic, HIV infection may be augmented with heightened immunosuppression, further complementing opportunism by the herpes group viruses.

**Herpes Simplex Virus (HSV)**

The prevalence of oral Herpes Simplex Virus (HSV) infection among HIV-positive patients has been reported to be between 5 and 20% (Silverman et al., 1986; Phelan et al., 1987; Melnick et al., 1989; Glick et al., 1994a). Nevertheless, while surprisingly uncommon, HSV infections tend to be particularly atypical, severe, and persistent, but rarely disseminate (Schiodt and Pindborg, 1987; Jones et al., 1992).

Few cases of genuine primary HSV infection (herpetic gingivostomatitis) arise as the prevalence of HSV antibodies among homosexual men and intra-venous drug users is high (seropositivity in 90-95% of cases) (Ficarra, 1997). The intra-oral lesions of HIV-infection are usually confined to the keratinised mucosa and begin as small crops of vesicles on the hard palate or gingiva, and occasionally on the dorsal surface of the tongue; these rupture to produce small painful ulcers.
The infection may progress rapidly in HIV disease and cause diffuse weeping ulcers that extend onto the facial skin (MacPhail et al, 1989). Severe mucocutaneous HSV disease does not develop in most AIDS patients, until the CD4+ lymphocyte count decreases to less than <100 cells mm$^{-3}$ (Bagdades et al, 1992).

**Varicella-Zoster Virus (VZV)**

Oral infections with Varicella-Zoster Virus (VZV) are not common in HIV-infection, though oral chickenpox, presenting as erythematous lesions and ulcers has been recorded (Schodt et al, 1987). The intra-oral lesions of VZV infection present as vesicles and ulcers, and may occur on any mucosal surface. The distribution of the ulcers is usually unilateral, following the distribution of the trigeminal nerve, with a characteristic change from affected mucosa to normal mucosa at the midline. The lesions may occasionally be preceded by complaints of pain, apparently emanating from the teeth in the absence of a dental cause.

Of significance, zoster in HIV-disease, is more common cranially than in non-HIV-infected persons, cutaneous dissemination is more frequent, and the clinical course may be more severe, with an increased risk of mortality (Murray et al, 1988; Morfeldt-Manson et al, 1989). One study demonstrated that, people who developed herpes zoster involving the trigeminal nerve had a more rapid progression to AIDS than those with zoster lesions elsewhere (Melbye et al, 1987).
Acyclovir systemically (orally or intravenously), is the most generally accepted therapy for these infections in HIV-positive persons (Table 2:2). HSV is however, sometimes resistant as a result of defective viral thymidine kinase; the incidence of acyclovir-resistant herpes simplex virus isolates after prolonged treatment has been estimated to be about 5% in immunosuppressed patients (Itin and Lautenschlager, 1997). Foscarinet, an inhibitor of DNA polymerase, may be an effective alternative (McPhail et al, 1989; Safrin et al, 1991).

Cytomegalovirus (CMV)

Oral mucosal involvement by Cytomegalovirus (CMV) in HIV-infection is uncommon. When infection of the oral tissues occurs, large, sharply demarcated oval ulcers lacking rolled margins, which are often multiple and painful, are present (Kanas et al, 1987; Jones et al, 1992; Heinic et al, 1993b; Schubert et al, 1993; Firth et al, 1994). Usually, these ulcers only occur in those with pre-existing CMV infection (Langford et al, 1990; Heinic et al, 1993b). Sometimes, these ulcers mimic periodontal infection (Glick et al, 1991; Dodd et al, 1993a). Ulcers co-infected with CMV and HSV have also been reported (Jones et al, 1992; Heinic et al, 1993b; Regezi et al, 1996; Flaitz et al, 1996). Central mandibular infection by CMV in HIV-disease has also been described (Berman and Jensen, 1990; Jones et al, 1993; Flaitz et al, 1994).

Accurate diagnosis requires histological examination of lesional tissues with appropriate immunohistochemistry. Examination of lesional tissue reveals
granulation tissue with typical CMV inclusions identifiable in endothelial cells and other connective tissue cells (Kanas et al, 1987). Antiviral agents such as, gancyclovir and foscarnet can be useful therapeutic agents.

Human Herpes Simplex virus 6 and 7 (HHV-6 and 7) are mentioned in the salivary gland disease section below, HHV-8 in the Kaposi’s sarcoma section below, and Epstein-Barr Virus (EBV), which is associated with oral hairy leukoplakia (OHL), is discussed in detail in Chapter 3.

**Human Papilloma Virus (HPV)**

Oral verruca vulgaris, condyloma acuminatum and focal epithelial hyperplasia, all caused by human papillomaviruses (HPV), have been reported to occur with a range from 0-5.3% in HIV-positive persons (Greenspan et al, 1988a; de Villiers, 1989; Barone et al, 1990; Lamster et al, 1994; Shiboski et al, 1994; Vilmer et al, 1994; Viraben et al, 1996). Clinically, HPV-associated lesions are papillary, sessile or pedunculated lesions or may be papular; Most oral condylomas are multiple and involve many mucosal sites.

The more common cauliflower and spiky warts can be due to various HPV types, including 2, 11, 13, 16, 18 and 32, as well as HPV 7, which was not seen in the mouth before HIV infection (Greenspan et al, 1988a; Syrjanen et al, 1989a; Volter et al, 1996). Regezi and co-workers (1994), documented HPV-associated epithelial atypia in oral warts of HIV-positive patients, however, malignant
transformation has not been seen (Greenspan and Greenspan, 1996), as, besides HPV-infection, interaction of several co-factors may be necessary for the induction of oral squamous cell carcinoma in HIV-infected patients (Flaitz et al, 1995a). Cryotherapy, laser or surgical excision are the usual treatments, although, there may be recurrences of the lesions, possibly caused by the presence of latent HPV infection, even in the clinically normal mucosa.

Molluscum contagiosum (MC)

Molluscum contagiosum belongs to the pox group of DNA viruses and is a self-limiting disease, affecting the skin and rarely the oral mucosa (Whitaker et al, 1991). It appears as single or multiple umbilicated papules, that may itch, leading to scratching and autoinoculation (Sugihara et al, 1990a). In HIV-infection, MC may be progressive, widespread and atypical (Itin and Gilli, 1994). In a report of 8 HIV-infected patients with facial and perioral MC, lesions on the upper and lower lip were recognized (Ficarra et al, 1994b). The appearance of large and numerous MC in HIV-infected patients indicates marked disease progression, and low CD4+ lymphocyte counts (Schwartz and Myskowski, 1992). Curretage and cryosurgery can be effective “bloodless” treatments, although recurrence is very likely.

Human Immunodeficiency Virus (HIV)
Oral lesions induced by HIV itself during symptomatic primary infection, include oral ulcerations and spotty erythemas (Kinloch-de Loes et al, 1993; Lapins et al, 1996).

Komitowski and co-workers (1993), demonstrated that subclinical HIV-associated changes in oral mucosa can be shown in an early stage with image analysis of tongue smears. They found marked differences in nuclear and chromatin features between HIV-infected patients and controls. In a further study, cytological tongue smears of HIV-infected patients showed increased expression of cytokeratins 10/11 and 19 and reacted positively for MHC class II antigens (Langford et al, 1992a). The significance of these findings is unknown.

In addition, significant reduction of Langerhan’s cells has been reported in oral mucosal washings from HIV-positive patients (Sporri et al, 1994). As Langerhans’s cells play a major role in the mucocutaneous immune system, the authors concluded that, the frequent occurrence of oral lesions in HIV-infected patients may, in part, be caused by the reduced Langerhan’s cell number and function.

**Bacterial Infections**

**Periodontal Disease**

The reported prevalence of HIV-associated periodontal diseases has ranged from 0% to 50% of HIV-seropositive subjects affected by HIV-associated gingivitis
(HIV-G), and from 0 to 22% of HIV-seropositive subjects affected by HIV-associated periodontitis (HIV-P) (Silverman et al, 1986; Phelan et al, 1987; Roberts et al, 1988; Barone et al, 1990; Friedman et al, 1991; Klein et al, 1991; Swango et al, 1991; Murray et al, 1989; Schulten et al, 1989; Porter et al, 1989; Tukutuku et al, 1990; Masouredis et al, 1992; Laskaris et al, 1992a; Gillespie and Marino, 1993; Riley et al, 1992; Smith et al, 1995). However, some of the previous studies may have over-estimated the incidence of HIV-associated periodontal diseases, as the clinical criteria for their diagnosis have varied, partly as a result of confusion and changes in nomenclature (Smith et al, 1993).

A report on the classification and diagnostic criteria for oral lesions in HIV-infection attempted to harmonise previous classifications of periodontal diseases, and eliminated the term HIV from the terminology (EC-Clearinghouse and WHO, 1993), although this was described by a group of non-periodontologists. This classification comprises:

**Linear gingival erythema (LGE)**, a distinct fiery red band along the margin of the gingiva, with disproportionate amount of erythema for the amount of plaque seen, without ulceration and evidence of pocketing or attachment loss.

**Necrotizing (ulcerative) gingivitis (NUG)**: destruction of one or more interdental papillae, with necrosis, sloughing, ready haemorrhage and characteristic foetor.

**Necrotizing (ulcerative) periodontitis (NUP)**: soft tissue loss as a result of ulceration or necrosis, with exposure, destruction or sequestration of bone, pain and loosening of teeth.
Necrotizing (ulcerative) stomatitis (NUS): a severe, often localized periodontitis or massive spreading destructive infection of gingiva, soft palate and associated bone, resembling noma (Williams et al., 1990; Winkler and Robertson, 1992). In addition, cancrum oris, caused by gram-positive bacteria and anaerobes has also been reported, in the oral cavity of an HIV-positive patient (Barrios et al., 1995).

Lamster and co-workers, (1997) estimated, that the prevalence of NUG and NUP in HIV-seropositive populations to be of 10% and 5% respectively.

The more severe forms of HIV-associated periodontal disease (NUG, NUP) are generally associated with pronounced immunosuppression (Lucht et al., 1991; Barr et al., 1992b), but not all individuals who are immunosuppressed will present with these lesions (Epstein et al., 1993a; Barr et al., 1996). Glick and co-workers (1994b), observed that, the mean CD4+ lymphocyte count in HIV-infected individuals with NUP was only 32 cell mm$^{-3}$ and the probability of death within 24 months of the diagnosis of NUP was 73%.

A positive correlation between HIV infection and rapid progression of periodontal disease was reported by Yeung and co-workers, (1993), explained by the local absence of effector and regulatory cells in HIV-positive patients (Steidley et al., 1992), although such observations have not always been reported (Smith et al., 1995; Odden et al., 1995). Recently, Robinson and co-workers (1996a), suggested that, HIV-seropositive homosexual men demonstrated significantly greater probing attachment loss, probing depth, bleeding on
probing, gingival ulceration, diffuse gingival erythema and punctate erythema compared to HIV-seronegative homosexual men.

The pathogenesis of HIV-related periodontal disease may involve a loss of helper T-cell activity (on which immunological response to the periodontal flora depends), polymorphonuclear cell defects, and the presence of periodopathogenic bacteria. Linear gingival erythema may represent hyperaemia due to vasoactive cytokines, rather than gingivitis (Greenspan and Greenspan, 1996). Cigarette smoking may be a co-factor in the HIV-associated periodontal disease (Swango et al, 1991).

The role of specific periodontal microflora is questionable, as several studies have suggested that, subgingival bacteria present in HIV-seropositive patients with and without periodontal disease is similar to that found in systemically healthy subjects with or without periodontal disease (Rams et al, 1991; Lucht et al, 1991; Piluso et al, 1993; Moore et al, 1993). However, some uncommon, often extra-oral pathogens have been found in the subgingival environment of HIV-seropositive patients such as, *Mycoplasma salivarium, Bacteroides fragilis, Fusobacterium varium, Fusobacterium necrophorum, and Enterobacter cloacae* (Rams et al, 1991; Moore et al, 1993), and although, these pathogens are detected in low levels, they may contribute to the unusual forms of periodontitis seen in the limited number of patients with severe disease (Rams et al, 1991). In addition, *Candida* species have been detected in the dental plaque of HIV-infected individuals (Murray et al, 1989; Zambon et al, 1990; Rams et al, 1991;
Moore et al., 1993; Odden et al., 1994), and an association between oral candidosis and LGE has been suggested (Grbic et al., 1995).

There is no specific therapy for HIV-related periodontal disease; currently employed regimens include: debridement of the lesions to remove slough, plaque, necrotic hard and soft tissue; oral metronidazole and chlorhexidine mouthwashes; irrigation with povidone iodine; meticulous oral hygiene, and follow-up to reinforce oral hygiene, root planning and removal of sequestra (Winkler and Robertson, 1992; Holmstrup and Westergaard, 1994).

Other Bacterial Infections

Odontogenic infections may occasionally persist or may spread in HIV disease (Vuillecard et al., 1989). Occasionally, but not always, extraction sockets become infected, heal slowly (Reichart et al., 1987; Watkins et al., 1991; Robinson et al., 1992; Porter et al., 1993) or are complicated by actinomycosis (Watkins et al., 1991).

Though, mycobacterial infections are common in HIV disease, there have been few reports of oral lesions, mostly caused by atypical mycobacteria such as, M. avium intracellulare, M. chelonae, M. kansasii (Volpe et al., 1985; Friedman et al., 1994; Nuesch et al., 1996; Robinson et al., 1996b).
Oral manifestations of *Treponema pallidum* infection in HIV-disease are associated with the presence of ulceronodular lesions (lues maligna) and white plaques or papules on an erythematous base, both corresponding to the secondary stage of *Treponema pallidum* infection (Shulkin et al., 1988; Gregory et al., 1990; de Rie et al., 1991; Ficarra et al., 1993a; Sands and Markus, 1995; Ramirez-Amador et al., 1996b).

Oral infections with gram-negative bacteria such as, *Klebsiella pneumoniae, Enterobacter cloaca*, *Escherichia coli, Pseudomonas aeruginosa, Salmonella enteritidis* are rare, but can present as oral ulcerations and/or redness (Indrisano and Simon, 1989; Felix et al., 1991).

*Leishmaniasis* caused by *Leishmania donovani infantum*, typically occurs in persons who have travelled in geographic areas, where leishmaniasis is endemic; however, oral lesions have been described in HIV disease (Michiels et al., 1994).

Epithelioid angiomatosis is a skin lesion, caused by a Gram-negative bacillus termed *Rochalimaea henselae* (Slater et al., 1992) or the closely related *Rochalimaea quintana* (Koehler et al., 1992), manifesting as pink or purple papulonodular lesions or plaques (Levell et al., 1995). Oral lesions can resemble Kaposi’s sarcoma (Speight et al., 1991; Glick and Cleveland, 1993; Monteil et al., 1994; Levell et al., 1995). The diagnosis of epithelioid angiomatosis is reliant on histology and appropriate staining of lesional tissue (Glick and Cleveland, 1993).
Unlike Kaposi’s sarcoma, the condition may respond to broad-spectrum antibiotics (Speight et al, 1991).

2. Neoplasms

Individuals with AIDS have approximately a 40% risk of developing a malignant disease during their lifetime; this risk is influenced by the mode of acquisition of HIV-disease (e.g., Kaposi’s sarcoma is more common in HIV-infected homosexual men), and is expected to increase with prolonged survival associated with improved medical care (Epstein and Scully, 1992).

Kaposi’s sarcoma (KS)

Classic Kaposi’s sarcoma (KS) is an indolent neoplasm of elderly men in or from Eastern and Mediterranean European countries (classic KS), manifesting as paranodular skin lesions with little or no visceral involvement (Oettle, 1962). It has also been recognized as a tumour endemic in parts of East and Central Africa (endemic KS), with rapid disease progression and systemic involvement (Martin et al, 1993). KS can also arise in relation to iatrogenic immunosuppression (Fife and Bower, 1996; Gotti and Remuzzi, 1997).

Kaposi’s sarcoma contains endothelial cells and prominent spindle-shaped cells with few mitoses, extravasated erythrocytes, and the presence of haemosidirin (Green et al, 1984; 1988; Kuntz et al, 1987; Regezi et al, 1993). Whether it is a
clonal neoplasm (Rabkin et al, 1995), or represents hyperplastic growth (Costa and Rabson, 1983) remains controversial; however, its metastatic behaviour suggests a malignant pathology.

In the first years of the HIV epidemic in San Francisco, KS occurred in about 50% of gay men with AIDS (Lozada et al, 1983; Silverman et al, 1986), although its prevalence has been decreasing during the recent past, occurring in approximately 20% of gay men with AIDS (Boshoff et al, 1997). Nevertheless, the frequency of KS in HIV disease is still strikingly higher in homosexual or bisexual males, than patients infected via blood and blood products, or heterosexual infection with non-bisexual males (Beral et al, 1992; Hermans et al, 1996), and may show distinct geographic distributions suggesting a clustering of disease (Beral et al, 1992). In addition, the classic KS may be more common in non-HIV-infected homosexual men than the general population, suggesting transmission of the HIV-related KS by an infectious agent (Beral et al, 1992).

The likely transmissible etiological agent for KS is Human Herpes Virus 8 (HHV-8). Human Herpes Virus 8 is related to EBV, and even more closely, to Herpesvirus saimiri (HVS) of the Gamma-herpesvirinae subfamily, both known to have oncogenic capabilities (Chang et al, 1994; Moore et al, 1996). Human Herpes Virus 8 sequences and transcripts are present in the nuclei of spindle cells and endothelial cells of all types of KS lesions, and also in circulating KS-like spindle cells, therefore, the virus is present in the representative neoplastic cells of KS (Boshoff et al, 1995; Aluigi et al, 1996; Zhong et al, 1996; Zhong and
Most importantly, detection of HHV-8 sequences in the blood of HIV-infected subjects without KS may be strongly predictive of the later development of KS. In one study, nearly 80% of HHV8-positive patients developed KS within 4 years of HHV 8 detection (Whitby et al, 1995). The virus has been detected in oral KS (Di Alberti et al, 1996; Jin et al, 1996; Flaitz et al, 1997), in non-KS oral lesions in HIV disease (Di Alberti et al, 1997a; 1997b), and also in saliva of up to 33% of HIV-infected persons, but not in HIV-negative individuals (Boldogh et al, 1996).

Human Immunodeficiency virus infection may contribute to the development of KS in two distinct ways. First, the immune deficiency caused by HIV, may increase the likelihood of opportunistic HHV-8 infection, and secondly, the tat protein of HIV may act synergistically with basic fibroblast growth factor, at least in culture, to stimulate the proliferation of endothelial cells (Ensoli et al, 1994). Currently, KS accounts for over 80% of all HIV-associated malignancies, and there is an increased risk for development of second malignancies in patients with KS. The development of KS also heralds progression of HIV infection, death occurring within a 14-month median time, and in the presence of an opportunistic infection, there may be only a 6-9 month median survival (Flaitz et al, 1995b).

Kaposi’s sarcoma is clinically characterised by multifocal, widespread lesions involving the skin, oral mucosa, lymph nodes and organs such as the gastrointestinal tract, lung, liver and spleen. At post-mortem, virtually all organs
including brain, pancreas, heart and major vessels may be found to be involved (Porter and Scully, 1994).

Oral and perioral KS is often an early manifestation of HIV disease (Dodd et al., 1991b), or AIDS (Mostofi et al., 1985). Indeed, the oral cavity may represent the initial site of KS, in up to 60% of the patients (Flaitz et al., 1995b). The oral lesions of KS typically present as red, bluish or purple, flat patches or macules, which become nodular or ulcerated at later stage (Lozada et al., 1983; Lumerman et al., 1988; NaPier et al., 1983; Eversole et al., 1983; Nickles et al., 1984; Ficarra et al., 1988a; Wescott and Werksman, 1989; Epstein and Scully, 1991; Reichart et al., 1993; Shiboski and Winkler, 1993; Ficarra and Eversole, 1994; Flaitz et al., 1995b), although there are reports of non-discoloured oral KS (Barrett et al., 1988; Daly et al., 1989; Reichart and Schiodt, 1989).

In flat, patch like-lesions there may be a marked increase of CD8+ T-cells compared to normal mucosa, with a reduction in their numbers in later tumour stages, while in both flat and exophytic KS the number of HLA-DR+ cells are higher than in uninvolved mucosa, reflecting the local influence of KS growth factors on the inflammatory reaction in the setting of systemic immunosuppression (Tabata et al., 1993).

The most common oral site of KS is the palate, followed by the attached gingiva and dorsum of the tongue, but KS may involve any mucosal site, including non-keratinized tissues, particularly when advanced (Petit et al., 1986; Epstein and
Silverman, 1992; Reichart et al, 1993). The lesions may be painful, haemorrhagic and when large, may interfere with speech, eating or oral hygiene.

The differential diagnosis of oral KS may include, for macular lesions: atrophic candidosis, median rhomboid glossitis, erythroplakia, amalgam tattoo, and ecchymosis; for elevated KS: pyogenic granuloma, minor salivary gland tumour, bacillary angiomatosis, vascular lesion, haematoma, melanoma, and lymphoma (Flaitz et al, 1995b). Drugs, such as ketoconazole and zidovudine can cause oral pigmentation, which although usually brown, should not be confused with the reddish or purple colour of KS lesions. KS may also present as cervical lymph node or salivary gland enlargement (Yeh et al, 1989), occasionally, as an intraosseous lesions (Langford et al, 1991a; Nichols et al, 1995), or may mimic periodontal infection (Chapple et al, 1992).

The prognosis of HIV-infected patients with KS depends on the degree of immunosuppression, rather than on the neoplastic proliferation and tumour load (Errante et al, 1991). There is a suggestion that oral mucosal lesions of KS are associated with a lower CD4+ T cell count, than those with skin lesions alone (Orfanos et al, 1995). Currently, the majority of patients with AIDS-associated KS, die of opportunistic infections, rather than as a consequence of their KS.

Recently, a clinical staging system for oral KS was proposed, which include estimation of the extent of local involvement, the character of the lesion from flat
to elevated and exophytic, assessment of regional involvement and involvement at multiple sites (Epstein, 1997).

The primary goal of treatment of oral KS is palliation, as no data show that treatment improves patient survival. Relief of pain, reduction of oedema, associated lymphadenopathy and functional and cosmetic factors remain the main indicators for treatment of oral KS. Occasional exophytic lesions may be considered appropriate for excision. Lesions with a pedicular attachment may be easily removed by carbon dioxide or argon laser (Schweitzer and Visscher, 1990) or surgical excision, and if the lesion is resected, as far as to where the surrounding oedema extends, it generally does not recur at the same site. Cryosurgery has been used, but KS often persists beneath the cryolesion (Tappero et al, 1991). Nevertheless, KS most often present as macular or broad-based exophytic lesions and may not be easily treated with local surgical approaches.

Intra-lesional therapy with vinblastine has been shown to provide effective palliation of oral KS (Epstein and Scully, 1989; Nichols et al, 1993, Moyle et al, 1993; Flaitz et al, 1995c; McCormick, 1996), although it produces pain for 1-3 days (Epstein and Scully, 1989; Epstein et al, 1989). Some patients may develop numbness at the site of palatal lesions, that may persist for 2-4 weeks, which is likely related to local effects of the drug. The use of sclerosing agents, such as, 3% sodium tetradeyl sulphate, has been shown to be effective (Lucatorto and Sapp, 1993), but mucosal ulceration and discomfort are considerable side effects,
which limit their use. Interferon-alpha has been reported to be beneficial clinically, but requires repeated injection (twice weekly) and the drug is expensive (Sulis et al, 1989; Safai et al, 1990).

When lesions involve a more extensive area, resulting in regional involvement, such as the entire hard palate and/or oropharynx, regional treatment provided by radiotherapy may be appropriate. While radiation therapy has been reported to result in a severe, painful and persisting mucositis, and may require hospitalization of the patient, increased numbers of fractions, rather than single fraction radiation may reduce the severity of the oral complications (Epstein and Silverman, 1992). The decision to treat with systemic chemotherapy depends upon systemic involvement, and must consider the general health of the patient, potential systemic side-effects of therapy and the risk of additional immunosuppression (Epstein and Scully, 1992).

Single agent chemotherapy may control the disease in approximately 30% of patients, while combination chemotherapy can produce transient responses in up to 93% of selected patients (Boshoff et al, 1997). Treatment has included vinblastine, vincristine, doxorubicin, bleomycin, alpha-interferon and other agents. The therapy always result in further suppression of the immune system, and interference or interaction with other medications (Boshoff et al, 1997).

KS may also regress with zidovudine (Langford et al, 1989a) or thalidomide therapy (Soler et al, 1996a).
Overall, treatment of AIDS-associated KS does not significantly affect the prognosis or survival of AIDS patients, however, treatment can alleviate aerodigestive and/ or respiratory dysfunction, allow for adequate nutritional intake, and improve the quality of life for these patients (Flaitz et al, 1995b).

**Non Hodgkin’s lymphoma (NHL)**

Approximately, 3% of patients will develop a lymphoma during the course of HIV-disease (Ficarra and Eversole, 1994). The most common type, non-Hodgkin’s lymphoma (NHL), is now the second most frequent neoplasm of HIV-disease after Kaposi’s sarcoma (Lozada-Nur et al, 1996). The development of lymphoma is a relatively late feature of HIV disease, and is considered as, an AIDS-defining event (Levine, 1992).

The vast majority (80-90%) of HIV-associated lymphomas are usually high-grade B-cell neoplasms, consisting of immunoblastic or small cell, non-cleaved lymphomas, which in individuals not infected with HIV, would account for only 10-15% of all lymphomas (Lowenthal et al, 1988). The HIV-related lymphomas tend to present at a younger age than in non-HIV-infected patients (Ziegler et al, 1984), and in up to 84% of affected individuals, there are multiple sites of extranodal involvement (CNS, heart, anus, rectum, adrenal glands and orofacial region (Levine, 1992). T-cell lymphomas do occur, albeit rarely, in HIV-disease (Lowenthal et al, 1988; Nasr et al, 1988; Lust et al, 1989).
Approximately, 50% of HIV-associated NHLs carry EBV genomes (Green and Eversole, 1989), unlike the NHLs of iatrogenic immunosuppressed patients, in which, 90% are EBV positive (Boshoff et al., 1997). The reasons for this discrepancy are not clear. HIV infection induces polyclonal B-lymphocytic activation, and the increased B cell proliferation alone may lead to further opportunities for cell transformation, c-myc translocation and the emergence of malignant clones without the involvement of an oncogenic virus (Boshoff et al., 1997).

Oral lymphomas, typically present in the fauces, gingiva, palate, or elsewhere, as a rapidly growing mass, an ulcer or tooth mobility (Silverman et al., 1986; Green and Eversole, 1989; Kaugars and Burns, 1989; Leess et al., 1987; Brahim et al., 1988; Rubin et al., 1989; Groot et al., 1990; Colmenero et al., 1991; Langford et al., 1991b; Donkor et al., 1991; Piluso et al., 1994; Gowdey et al., 1995a; Zapater et al., 1996).

Oral lymphomas can be the first sign of HIV disease (Berberi et al., 1995; Nittayananta et al., 1996), or AIDS (Lozada-Nur et al., 1996; Wolvious et al., 1997), but usually are diagnosed as secondary events. HIV-related oral lymphoma may present as ulcers on several mucosal surfaces (Dodd et al., 1993b), as delayed healing of an extraction wound (Nittayananta et al., 1996), as lesions which disappeared and then reappeared (Dodd et al., 1992), or even mimic odontogenic infection (Hartwig et al., 1995). Occasional cases of Hodgkin’s disease (Laskaris et al., 1992b), Burkitt’s lymphoma (Hernandez
Vallejo et al, 1989), anaplastic large lymphoma (Ki-1) (Hicks et al, 1993; Willard et al, 1995) and T-cell lymphoma (Thomas et al, 1993), involving the oral mucosa, have been reported, while recently a series of oral B-cell lymphomas with unique immunohistologic features, called plasmablastic lymphomas, were reported by Delecluse and co-workers (1997).

Diagnostic investigations include: fine needle aspiration, chest radiography, CT or MRI scanning, gallium scanning, ENT examination, bone marrow biopsy, lumbar puncture, immunostaining or cytogenic examination of tumour cells (Armitage, 1993; Porter and Scully, 1994).

The management of oral lymphomas of HIV disease typically requires radiotherapy, unless there is widespread lymphomatous disease, when chemotherapy is also warranted (Epstein and Scully, 1992; Epstein and Silverman, 1992). Currently, the median survival of patients with AIDS-related lymphomas after treatment is only 5-11 months (Ficarra and Eversole, 1994; Carbone et al, 1995). The diminished response to chemotherapy in patients with AIDS, may be due to factors such as, delay in diagnosis, the prevalence of high-grade microscopic subtypes, impaired haematopoietic function associated with HIV infection and high susceptibility to opportunistic infections because of the progressive immunodeficiency (Lozada-Nur et al, 1996).

**Oral squamous cell carcinoma**
Although oropharyngeal squamous cell carcinoma has been reported in patients with HIV (Silverman et al, 1986; Epstein and Scully, 1992; Tenzer et al, 1992; Reynolds et al, 1993; Roland et al, 1993; Flaitz et al, 1995a), it is a rare occurrence. Recently, a study reported putative risk factors for the development of oral squamous cell carcinoma in HIV-infected individuals, such as, immune dysfunction, viral agents, invasive factors, proliferating cell nuclear antigens, as well as, typical risk factors like tobacco and alcohol use (Flaitz et al, 1995a). However, in view of the small number of reported patients with HIV-related oral squamous cell carcinoma, HIV disease cannot be regarded as a risk factor for oral tumours other than KS and lymphoma.

3. Other Lesions

Oral Ulceration

Oral ulcers occur in 2 to 15% of HIV-infected patients (MacPhail et al, 1992; Piluso et al, 1996). The ulcers can cause profound pain and morbidity, interfere with speech and swallowing, and subsequently, adversely affect the nutritional intake, general health and quality of life of affected persons (Chaudhry et al, 1996; Ficarra, 1997).

The HIV-associated oral ulceration can be categorized into two general groups. Those ulcers caused by a specific organism (eg. as a result of viral, fungal, bacterial or protozoan infection) or associated with a neoplastic process
(Kaposi’s sarcoma, non-Hodgkin’s lymphoma, squamous cell carcinoma), and those ulcers, where none of the aforementioned aetiologies are identified, the histologically 'non-specific' ulcers.

Ulcers in the first group are diagnosed by identification of the causative organism or neoplastic process, and are treated accordingly. Those in the second group are diagnosed, in part, by the absence of any of the causal organisms or processes of the first group. This second group can be further divided on the basis of the clinical appearance of the ulcers; location on keratinized and/or non-keratinized mucosa, pattern of recurrence (recurrent or persistent), and variations in treatment response (successful or unsuccessful corticosteroid therapy). Included in this group are atypical ulcers, recurrent aphthous ulcers (RAU), non-specific or 'aphthous-like' ulcers, neutropenic ulcers, medication-induced ulcers, traumatic ulcers and ulcers caused by unidentified pathogens.

According to the revised WHO classification of oral manifestations of HIV infection (EC-Clearinghouse and WHO, 1993), the atypical idiopathic ulcers have been termed “ulcers, not otherwise specified (NOS)”.

Painful recurrent aphthous ulceration (RAU), may be seen in HIV-infection (MacPhail, 1991; MacPhail et al., 1992; Reyes-Teran et al., 1992). In contrast to recurrent intra-oral herpes simplex, RAU occur on non-keratinized mucosa, such as, the lateral margins of the tongue. Minor RAU, appear as ulcers 2-5 mm in diameter, covered with pseudomembrane, and surrounded by an erythematous
halo. Major RAU are large, over 1 cm in diameter; they may persist for months and cause pain, impairment of speech, and difficulty in swallowing. Herpetiform RAU are 1-2 mm in diameter and occur in crops.

Whereas, the prevalence of RAU in HIV-infected persons may not be increased, their severity may be markedly enhanced. Some reports suggest that, there may be a recurrence in HIV-positive individuals, who have not experienced ulcers for many years (MacPhail et al, 1991; Phelan et al, 1991). In addition, the number and type of lesions present, and the frequency and duration of episodes may be increased in HIV-disease; furthermore, lesions may occur in more bothersome locations, such as, the soft palate or tongue rather than the buccal or labial mucosa (Phelan et al, 1991; Mac-Phail et al, 1992; Muzyca and Glick, 1994; Piluso et al, 1996).

Previous studies have shown, in otherwise healthy subjects, depressed or reversed CD4+:CD8+ ratio, increased adhesion molecule expression (ELAM, ICAM-1 and CD18), increased number of γδ TcR T cell and increased production of TNF-α, in the peripheral blood of patients with active lesions, compared with controls or with patients with inactive ulcers (MacPhail and Greenspan, 1997). Thus, similar HIV-related changes in the immune system may exacerbate pathogenic mechanisms of RAU, and contribute to their severity.

These RAU-like ulcers may first appear during HIV-seroconversion, and can be associated with a cutaneous rash and pharyngeal and/or esophageal ulcers
(Rabeneck et al, 1990). Major aphthous ulcers have been suggested by some workers to be markers of progression of HIV disease (Muzyca and Glick, 1994).

Persistent ulcers may require biopsy to exclude possible opportunistic infections or malignancies. RAU-like ulcers may respond to topical corticosteroids and tetracyclines (Phelan et al, 1991; MacPhail et al, 1992), thalidomide (Youle et al, 1989; Radeff et al, 1990; Ghigliotti, et al, 1993; Paterson et al, 1995; Thompson, 1995; Weidle, 1996; De Vincenzo and Burchet, 1996; Soler et al, 1996b; Ball et al, 1997; Jacobson et al, 1997) or systemic corticosteroids (MacPhail, 1992; Reyes-Teran et al, 1992; Silverman et al, 1992; de Asis et al, 1995). Systemic corticosteroids should not be the agents of first choice, as they may cause deterioration. In addition, resolution of HIV-associated atypical ulceration, after restoration of neutrophil count with granulocyte-colony-stimulating factor (G-CSF) has recently been reported (Luzzi and Jones, 1996).

Oral ulcers in HIV-disease can also be due to medication, such as, 2, 3-dideoxycytidine, zidovudine, foscarnet and chemotherapeutic agents given for KS or NHL, such as bleomycin, adriamycin, vincristine and vinblastine (Ficarra, 1992; 1997). Radiotherapy for oral KS and NHL may also be responsible for severe mucositis, and bone necrosis (Ficarra and Eversole, 1994). Severe reactions, such as, Stevens-Johnson syndrome and toxic epidermolysis have been observed after the administration of ketoconazole and sulphonamides (Rzany et al, 1993). Rare disorders presenting as oral ulcers such as, Behcet's disease,
Reiter's syndrome, necrotizing vasculitis and lymphomatoid granulomatosis have also been observed in patients with AIDS (Ficarra, 1997).

**Salivary Gland Disease**

HIV-Salivary gland disease (HIV-SGD) includes xerostomia and/or swelling of the major salivary glands, most commonly affecting one or both parotid glands and less frequently the other glands (Schiodt et al, 1989a; Schiodt et al, 1989b; Schiodt, 1992, 1995). The prevalence of HIV-SGD is in the range of 2-10% for xerostomia and approximately 1% for salivary swellings (Roberts et al, 1988; Porter et al, 1989; Schulten et al, 1989; Moniaki et al, 1990; Tukutuku et al, 1990; Ramirez et al, 1990; Schiodt, 1992; Fox, 1992; Shiboski et al, 1994).

The etiology of HIV-SGD may be multifactorial. HIV does not appear to play a direct role in this disease, as it has not been demonstrated by immunohistochemistry in the salivary gland epithelial cells (Schiodt, 1992). Greenberg and co-workers (1995;1997), found xerostomia, in HIV disease, to be related to CMV infection, while HHV-6, HHV-7 and HHV-8 have been detected in saliva and salivary glands of HIV-infected individuals (Di Luca et al, 1995; Boldogh et al, 1996); their role in the development of HIV-SGD is yet unknown. HIV-SGD may occur more frequently in individuals with an HLA haplotype containing HLA-DR 5, DR6 and DR7 (Schiodt, 1997).

The salivary gland swellings in HIV disease are usually diffuse and soft, enlarge slowly, but can increase to such a significant size that, some patients request removal of the affected gland for aesthetic reasons (Schiodt, 1992; 1995).
The complaint of xerostomia is often made at an early stage, and may occur in the absence of salivary gland enlargement (Schiodt et al, 1989a). The xerostomia of HIV-SGD can be confirmed by establishing diminished parotid and submandibular salivary flow rates; sialochemistry may also be helpful (Mandel and Reich, 1992; Schiodt et al, 1992). Nevertheless, the histological and sialochemical changes of HIV-SGD are similar to those of Sjogren’s syndrome (Atkinson et al, 1989; Mandel and Reich, 1992; Schiodt et al, 1992), although, it appears to be distinct from classic Sjogren’s syndrome, in that, serum autoantibodies Ro (SS-A) and La (SS-B) are lacking (Atkinson et al, 1993).

Some patients develop cystic salivary gland lesions similar to cystic benign lymphoepithelial lesions, and also cervical lymphadenopathy (Rubin et al, 1991; Terry et al, 1991; Mandel and Reich, 1992; Shaha et al, 1993; Calderon-Osuna et al, 1995; Martinoli et al, 1995). Occasionally, the presence of these lesions may lead to the diagnosis of the underlying HIV disease (Albrecht et al, 1996). Interestingly, one study has demonstrated active replication of HIV-1 within dendritic reticular cells of cystic lymphoepithelial lesions of the parotid (Labouyrie et al, 1993). Recently, a case of bilateral multiple parotid calculi, which are uncommonly diagnosed in the normal population, was described by Ottaviani and co-workers (1997), in a patient with AIDS.

HIV-related SGD is associated with a CD8+T cell lymphocytosis, which may explain why, the prognosis for patients with SGD appear more favourable than
for other HIV-infected patients, possibly due to the inhibiting effect of CD8+T-cells on HIV (Kazi et al., 1996).

Biopsy of the major and minor glands, labial gland biopsy, sialography, fine needle aspiration, ultrasonography, CT scanning, magnetic resonance imaging and gallium scanning, can be useful in the diagnosis of HIV-SGD, although salivary scintiscanning with technetium pertechnetate appears to be of little value (Schiodt et al., 1989a; Itesku et al., 1990; Marmary et al., 1990; Terry et al., 1991; Rubin et al., 1991; Scully et al., 1993).

Zidovudine may produce regression of symptoms of HIV-SGD and the associated salivary gland swelling (Schiodt et al., 1992). Xerostomia can be treated with conventional measures (Silverman, 1989). Cystic lesions may respond to chemotherapy (Albrecht et al., 1996), low dose radiotherapy (Beitler et al., 1995) or enucleation (Ferraro et al., 1993). Superficial parotidectomy is indicated for discrete solid swellings (Huang et al., 1991).

**Oral purpura**

Oral manifestations of thrombocytopenia arise in 1-11% of HIV-infected patients and include petechiae, ecchymoses, a bleeding tendency with spontaneous gingival haemorrhages or postoperative bleeding (Reichart et al., 1987; Schiodt and Pindborg, 1987; Schulten et al., 1989; Rossi et al., 1990; Moniaki et al., 1990).
HIV-related thrombocytopenia is possibly related to HIV infection of megakaryocytes, as these cells express CD4 receptor, and may thus be a target of HIV (Schiodt, 1997).

Platelet transfusions and/or corticosteroids are sometimes indicated, for patients requiring dental surgery, particularly pre-operatively (Silverman, 1989). However, the risk of postoperative bleeding after dental extractions in non-haemophiliac HIV-infected patients in general, is not significantly higher than among HIV-negative controls (Porter et al, 1993).

**Oral hyperpigmentation**

Oral hyperpigmentation, in the form of brownish or brown-black macular discolouration has been described in up to 7% of HIV-infected patients (Schiodt and Pindborg, 1987; Langford et al, 1989b; Porter et al, 1989; Ficarra et al, 1990; Moniaki et al, 1990; Ramirez et al, 1990; Barone et al, 1990). The oral pigmentation may be associated with hyperpigmentation of the skin and/or nails (Barone et al, 1990; Ficarra et al, 1990). Oral hyperpigmentation is typically associated with intraleukocytic melanin or pigment in the basal cell layer or lamina propria with premature melanosomes (Schiodt, 1997).

Often the aetiology is unknown, but identified causes include zidovudine (Tadini et al, 1991) or ketoconazole therapy (Langford et al, 1989b), adrenocortical
insufficiency secondary to probable adrenal *Mycobacterium avium-intracellulare* infection (Porter et al, 1990) or following radiation therapy for Kaposi’s sarcoma (Piccinno et al, 1995).

**Drug reactions**

Bilateral reticular keratosis or atrophic changes of the oral mucosa, histologically similar to drug-induced lichenoid lesions, after the administration of zidovudine or ketoconazole, in HIV-infected patients, were reported by Ficarra and co-workers (1993b).

Erythema multiforme has been noted following the administration of a number of drugs, including, fluconazole (Gussenhoven et al, 1991), didanosine (Parneix-Spake et al, 1992), antituberculous chemotherapy and trimethoprim-sulfamethoxazole (Bayard et al, 1992). Toxic epidermal necrolysis (TEN) is considered by some to be a severe form of erythema multiforme, manifesting as widespread cutaneous sloughing, oral mucosal ulcerations and crust formations on the lips; however, in HIV-infection, it is rare (Reichert et al, 1987).
Cranial neuropathies

HIV may affect the central nervous system (CNS), giving rise to neurologic disturbances. These include dementia, neuropathy and other signs. Similarly, opportunistic infections or neoplasms involving the central nervous system secondary to HIV infection may give rise to symptoms (Scully et al, 1991b).

In most studies of the oral manifestations of HIV infection, facial palsy is not mentioned. However, in one study from Africa, facial palsy was the first clinical sign of HIV infection (Belec et al, 1988). Other cases of HIV-related facial palsy were reported by Piette et al, 1986; Langford-Kuntz et al, 1988; Schielke et al, 1989; Howlett et al, 1989; Doutre et al, 1992; Ficarra, 1992; Mastroianni et al, 1994; Durham et al, 1993). The condition typically resolves spontaneously.

Neurologic symptoms involving the trigeminal nerve, may occur in HIV-infection, in association with opportunistic infection or neoplasms affecting the CNS (Pindborg and Reichart, 1995). Bilateral mental anaesthesia, sudden, generalized, dental pain and diplopia as the first symptoms of AIDS have been reported (Milam et al, 1986).

Miscellaneous

Granuloma annulare is an uncommon inflammatory disorder, of unknown aetiology, manifesting as generalized papular eruptions in an annular
configuration, which may resolve spontaneously. A papular lesion has been described in the buccal mucosa of an AIDS patient (Green et al, 1989a).

**Exfoliative cheilitis**, a chronic inflammatory disorder of the vermillion border of the lips, characterized by the persistent formation of scales and crusts, has been observed in a small group of HIV-positive patients (Phelan et al, 1987; Schulten et al, 1989; Porter et al, 1989; Laskaris et al, 1992c; Ramirez-Amador et al, 1993). Recently, an association with *Candida* infection was observed, suggesting that exfoliative cheilitis may be considered another variant of oral candidosis in AIDS patients (Reichart et al, 1997).

**Oral aspects of HIV infection in children**


However, oral lesions have been shown to be one of the earliest and most reliable indicators of pediatric HIV infection, as they are in adults (Ramoz-Gomez, 1997). In a recent study of 276 children (Ramos-Gomez et al, 1996), it was shown that, HIV-infected children are 15-25 times more likely than non-infected children to develop orofacial manifestations, especially oropharyngeal candidosis. In combination with other markers of disease progression, such as
CD4+ T-cell counts, the presence of oral manifestations in HIV-infected children less than 1 year of age, was a significant indicator of a child’s prognosis and a crucial factor in the planning of interventions and treatments (Ramos-Gomez et al, 1996).

Oral candidosis is the most common lesion in children, and often the first manifestation of HIV infection, with the most commonly reported type, the acute pseudomembranous variety (Ketchem et al, 1990; Moniaki et al, 1993; Del Toro et al, 1996).

Parotid swelling occurs in 20-47% of HIV-infected children, and it has been associated with a slower progression to death (Katz et al, 1993). Oral hairy leukoplakia is rare in children, as six cases have been reported so far (Greenspan et al, 1988b; Nadal et al, 1992; Ferguson et al, 1993; Laskaris et al, 1995). The most common HIV-associated malignancies in adults (Kaposi’s sarcoma and non-Hodgkin’s lymphoma), have never been associated with HIV infection in children (Ramos-Gomez, 1997). Periodontal diseases (LGE, NUG, NUP, NUS), recurrent herpetic and aphthous ulceration, are also frequently seen in children (Katz et al, 1993). A decline in CD4+ lymphocyte counts has been associated with an increasing severity of periodontal disease (Howell et al, 1996). An increased caries rate (Madigan et al, 1996), and delayed dental development (Del Toro et al, 1996) have also been reported.
CHAPTER 3

ORAL HAIRY LEUKOPLAKIA: AN EPSTEIN-BARR VIRUS ASSOCIATED ORAL LESION
Introduction

Oral hairy leukoplakia (OHL) is one of the many new disease entities brought to light by the HIV epidemic. Although innocuous, it is an early clinical marker of HIV infection, and is associated with poor prognosis in and increased severity of HIV disease. It is not exclusive to HIV-infected people, being also observed, albeit much more rarely, in other patients, mainly those with immunosuppression. OHL is the only biological entity in which Epstein-Barr virus (EBV) replicates floridly in vivo. While the relationship between EBV infection and the development of OHL remains undefined, many studies have been made that provide insight into the intriguing virus-host relationship that underlies OHL.

Clinical features

Typically, OHL manifests as adherent whitish or grey patches on the lingual lateral margins. To a lesser extent, it may also arise from the dorsum or ventrum of the tongue (Greenspan et al, 1984; Eversole et al, 1986; Schiodt et al, 1987a; Ficarra et al, 1991a). The surface of OHL is usually irregular, being thrown up into prominent folds or projections, sometimes so marked as to resemble “hairs”, but more commonly giving rise to a corrugated or shaggy appearance, hence its name (Greenspan and Greenspan, 1989). Occasionally, the lesion can be flat, particularly when sited at the ventral surface of the tongue (Schiodt et al, 1987a; Ficarra et al, 1991a). Less developed lesions can be detected as barely discernible
white areas on the postero-lateral lingual borders. OHL may also occur (albeit more rarely) on the buccal mucosa, floor of the mouth, soft palate, and even more rarely the oropharyngeal mucosa (Greenspan et al, 1984; Eversole et al, 1986; Sciubba et al, 1989; Kabani et al, 1989; Alessi et al, 1990; Ficarra et al, 1992). OHL is hence, regarded as primarily an oral mucosal lesion (Hollander et al, 1986). On rarer occasions, however, the esophagus may be affected (Logan et al, 1990). Although usually asymptomatic (Reichart et al, 1989), it may cause soreness (Greenspan et al, 1984), or a burning sensation (Schofer et al, 1987; Herbst et al, 1989). Furthermore, the unsightly appearance can be a source of concern (Greenspan and Greenspan, 1989). Table 3:1 summarizes the clinical features of OHL.

**Histological features**

Epithelial hyperplasia with hyperparakeratosis and acanthosis are consistent features of OHL (Eversole et al, 1986; Ficarra et al, 1991a). The thickened surface layer may separate from the underlying cells, giving rise to projections that produce the characteristic folds or “hairs” (Greenspan and Greenspan, 1989). Varying numbers of swollen, ballooned cells, with pyknotic nuclei and perinuclear haloes are usually present in the prickle cell layer, and occasionally in the suprabasal cell layer (Greenspan et al, 1984; Schiodt et al, 1987a). These koilocytes of OHL superficially resemble those in human papillomavirus (HPV) infection. However, while the nuclei of HPV-infected ballooned cells are enlarged and hyperchromatic, and the perinucleolar cytoplasmic halo is clear, the
nuclei of ballooned cells of OHL are small and pale, and the cytoplasm adopts a
glassy appearance (Syijanen et al, 1989b). In OHL, the vacuolated keratinocytes
demonstrate characteristic margination of the nuclear chromatin against the
nuclear membrane ("nuclear beading"). The latter histopathologic feature is
highly suggestive of OHL, and has been thought to result from rampant EBV
replication in the nucleus, which displaces chromatin to the nuclear margin. In
addition, three other nuclear changes have been described in OHL: an
eosinophilic central Cowdry type A appearance with a halo; a homogenous
eosinophilic or basophilic appearance with marginal chromatin clumping; and an
inclusion-type morphology in which the nuclei appear steel-gray or ground-glass
and the chromatin is marginated and clumped (Fernandez et al, 1990).

Candidal hyphae and leukocytes surrounding the hyphae are very frequently
observed in OHL lesions (Greenspan et al, 1984; Greenspan et al, 1985; Belton
and Eversole, 1986; Schiodt et al, 1987a; Kanas et al, 1988a; Kanas et al,
1988b). Bacterial colonies are commonly present in the parakeratotic areas
(Schiodt et al, 1987a; Reichart et al, 1989; Fowler et al, 1989b). Strikingly,
there is a paucity or absence of Langerhan's cells in OHL lesions (Daniels et al,
1987; Sciubba et al, 1989), and the inflammatory cell infiltrate in the lamina
propia is sparse (Araques et al, 1990; Southam et al, 1991). The histological
features of OHL are summarized in Table 3:2.
Pathogenesis

Although candidal hyphae are abundantly found in OHL lesions, and *candida* can be isolated from up to 70% of the lesions (Greenspan et al., 1984; Greenspan et al., 1985; Schiodt et al., 1987a; Ficarra et al., 1988b), OHL does not resolve with aggressive antifungal therapy, even when there is fungal clearance (Greenspan et al., 1984). While an etiologic link between *candida* infection and OHL is unlikely, there is evidence for the potentiating role of candidal co-infection (Boulter et al., 1996). An etiologic association with HPV was initially suggested (Greenspan et al., 1984). However, this has not been confirmed by detailed studies using electron microscopy (EM) (Belton and Eversole, 1986; Kanas et al., 1988b; Ficarra et al., 1988b; Resnic et al., 1988; el-Labban et al., 1988; Alessi et al., 1990; Manca et al., 1990), immunostaining (Kanas et al., 1988a; Resnic et al., 1988; Sciubba et al., 1989), and DNA hybridisation (Sciubba et al., 1989; Felix et al., 1993).

The frequent appearance of OHL in HIV-infected people, more commonly than in other patients who are immunosuppressed, points to HIV as playing some role in its induction. Nevertheless, most studies have failed to demonstrate genome in OHL lesions (Greenspan et al., 1987; Sciubba et al., 1989). More recently, however, HIV cDNA was reported in superficial squamous cells of OHL (Brandwein et al., 1996) and oral epithelial cells of HIV-infected people (Qureshi et al., 1995); the significance of these findings is unknown.
The relationship between EBV and OHL is intimate, the singular feature being the vegetative replication of EBV in the lesions. The evidence comes from many sources. EBV antigens associated with the replicative cycle (of the viral capsid antigen and membrane antigen complexes) have been demonstrated in tissue sections by immunohistochemistry (Greenspan et al., 1985; Zhang et al., 1988; Reichart et al., 1989; Sandvej et al., 1992) and EBV DNA by Southern blotting (Greenspan et al., 1985; Sciubba et al., 1989), in situ hybridization (ISH) (Greenspan et al., 1985; Loning et al., 1987; Syrjanen et al., 1988; De Souza et al., 1989; Sciubba et al., 1989; McClintock et al., 1991; Cubie et al., 1991; Felix et al., 1993; Mabruk et al., 1996) and PCR (Snijders et al., 1990; Mabruk et al., 1994). Intracellular herpes virions have been observed using thin-section EM (Greenspan et al., 1984; Lupton et al., 1987; Ficarra et al., 1988b; Zhang et al., 1988; Reed et al., 1988; Sciubba et al., 1989; Fowler et al., 1989b; Greenspan et al., 1989a; Araques et al., 1990; Manca et al., 1990; el-Labban et al., 1990; Kratochvil et al., 1990; Sugihara et al., 1990b; Itin et al., 1994). Further, transcripts expressed from the EBV BHLF1 and BCRF1 genes, which in cultured lymphoid cells are associated with the lytic replication cycle, have also been demonstrated (Ryon et al., 1993).

Additionally, experiments have shown that, the acanthosis and hyperplasia of OHL are directly related to the combined action of EBV-encoded proteins. Transgenic mice expressing the EBV BNLF-1 gene product, latent membrane protein (LMP)-1, develop lingual epithelial hyperplasia which is associated with expression of the hyperproliferative keratin 6 (Wilson et al., 1990). BHRF1, an
immediate early protein which bears homology to the anti-apoptotic Bcl-2 oncogene, is expressed in OHL tissues (Horner et al, 1995), and when transfected to a squamous cell carcinoma line, induces delay of terminal differentiation (Dawson et al, 1995). Thus, EBV has in OHL developed an effective strategy of permitting host cells to support very intense viral replication without their undergoing lysis.

How EBV infection initiates OHL has not been resolved. As the distribution of EBV DNA staining intensity resembles that of HPV in cervical epithelium, it was initially thought that, OHL arises from activation of latent EBV in the basal epidermal layer. Immunohistologic studies have demonstrated that antigens associated both with viral replication (including the BZLF-1 or ZEBRA protein) and latency (Epstein Barr virus nuclear antigens (EBNA) 1 and 2, and LMP-1) (Young et al, 1991; Niedobitek et al, 1991; Thomas et al, 1991; Rabanus et al, 1991; Palefsky et al, 1996; Ogutcin-Toller, 1996; Murray et al, 1996) are indeed expressed in a differentiation-associated pattern, with the staining being more intense towards the more superficial epidermal layers. However, these antigens, and EBV DNA as detected by ISH, are not observed in the basal layers (Young et al, 1991; Niedobitek et al, 1991; Thomas et al, 1991). Taken together, these findings are consistent with OHL resulting from superficial infection of cells in the spinous layer rather than activation of latent virus.

Saliva is a rich source of virus that could act as the transmission vehicle (Rickinson et al, 1985); past studies have shown that following primary EBV
infection, the virus continues to be shed from oropharyngeal epithelial cells (Sixbey et al., 1984) and the salivary glands (Wolf et al., 1984). Furthermore, a number of immunohistologic studies attempting to localize the EBV epithelial receptor, using the monoclonal antibody HB5, have failed to demonstrate the receptor in basal cells of the oral mucosa; rather, it is expressed in a differentiation-dependent manner from the mid-spinous layer (Corso et al., 1989; Talacko et al., 1991). The further observation that EBV encoded RNAs (EBERs), which are very sensitive markers of latent infection, are absent in OHL (Gilligan et al., 1990; Barletta et al., 1993) also supports the notion that reactivation does not play a part, and accords with other studies showing the B-cell as the predominant, if not exclusive, reservoir of latent EBV (Yao et al., 1989; Tao et al., 1995; Anagnostopoulos et al., 1995).

Nevertheless, other data support the activation-from-latency model. Becker and co-workers (1991), demonstrated BZLF-1 in the cytoplasm of basal epithelial cells in both HIV-infected patients and healthy carriers of EBV. Brandwein and co-workers (1996), showed that EBNA-2, LMP-1 and BZLF-1, can be found in basal and parabasal lingual cells. Significantly, the same authors demonstrated the basal location of EBV DNA by in situ PCR but not conventional in situ hybridization, suggesting that it is the relative insensitivity of ISH techniques used by previous workers that accounted for the absence of basal EBV DNA.

The recent report by Baskin and co-workers, (1995) of the proliferation of rhesus EBV in simian immunodeficiency virus (SIV)-infected rhesus monkeys provides
another insight into the pathogenesis of OHL. Epithelial lesions histologically resembling OHL, in which replicating rhesus EBV was found, were observed in the mucous membranes of the tongue, esophagus, penis, and the skin of SIV-infected monkeys. The absence of predilection for the oral mucosa in these lesions strikingly contrasts with the site-specificity of OHL, and suggests that the rhesus EBV-induced lesions arose either from viral activation following immunosuppression or haematogenous spread.

Extensive molecular studies demonstrate the multiplicity of EBV strains in OHL (Patton et al, 1990; Walling et al, 1992; Walling et al, 1994; Walling and Raab-Traub, 1994; Walling et al, 1995). Both genotypes, EBV-1 and 2, may be present within a single lesion (Labandeira et al, 1994) and the infecting types may change, with acquisition or loss of a type (Walling et al, 1992). Deletions, intertypic and interstrain recombinations also occur. Despite such findings, the role these newly created strains may play in the development of OHL is unclear. The discovery of one particular defective strain in OHL lesions, termed WZhet, which results from juxtaposition of the BamHI W and Z fragments of the EBV genome, was thought to play a key role in the initiation of viral replication (Patton et al, 1990). This recombinant genome encodes the BZLF1 or ZEBRA protein, which in lymphoid cells switches EBV from latency to undergo lytic replication (Miller et al, 1984; Rooney et al, 1988). However, the importance of WZhet has been weakened by the finding of its presence in non-OHL oral lesions (Ryon et al, 1993).
The potentiating role of cigarette smoking has been suggested by at least two studies (Boulter et al., 1996; Conley et al., 1996). Surprisingly, alcohol consumption appears to exert a protective effect (Boulter et al., 1996). The disparate outcomes of these two activities may relate to their opposing effects on epithelial differentiation (Boulter et al., 1996).

None of the findings outlined so far explains the predilection for OHL to arise from the lateral margin of the tongue. Some proposals have been made, eg. masticatory trauma (Thomas et al., 1991), but they remain unsubstantiated. Williams and co-workers, (1991) in observing that the lateral lingual border is the site of transition between the non-keratinized epithelium of the ventrum and the keratinized epithelium of the dorsum, suggested that the lability of cells at this junction somehow predisposes to EBV infection/activation; this interesting notion is yet to be substantiated. The effect of oral sex was examined in one longitudinal study, and found not to potentiate EBV replication in the tongue (Boulter et al., 1996).

**Clinical significance**

OHL can be an early, if not, the first sign of HIV infection (Schiodt et al., 1990; Lifson et al., 1994). Its appearance in all groups at risk of HIV (Eversole et al., 1986; Greenspan et al., 1986; Rindum et al., 1987; Schiodt et al., 1987b; Ficarra et al., 1988b), including children (Greenspan et al., 1988b; Nadal et al., 1992;
OHL is also associated with the subsequent rapid development of AIDS (Greenspan et al, 1987; Morfeldt-Manson et al, 1989; Moniaki et al, 1990; Katz et al, 1992; Maden et al, 1994; Ramirez-Amador et al, 1996a). In a study of 737 patients (principally comprising intravenous drug users) in Italy, the probability of developing AIDS in patients with OHL was 0.38 at 15 months, 0.64 at 25 months and 0.82 at 37 months, compared, respectively to 0.29, 0.52 and 0.78 in patients with oral candidosis (Moniaki et al, 1990). A study of 3 cohorts of homosexual and bisexual men in San Francisco showed that, while the mean time to AIDS was 1182 days for those with normal findings at baseline oral examination, that for those with OHL, oral candidosis and both oral lesions was 1032, 219 and 598 days, respectively (Katz et al, 1992). Nevertheless, Greenspan and co-workers, (1991), observed that 27 of a cohort of 198 HIV-infected patients with OHL did not develop AIDS during a follow-up period of 3 to 6 years, suggesting that the appearance of OHL is not an absolute ominous sign. Not considered in such studies is the possibility that EBV-DNA positivity in the oral epithelium, rather than clinically overt OHL, may be an earlier and more sensitive predictor of progression to AIDS (Nahe et al, 1991; Mabruk et al, 1995).

OHL can be used as a convenient clinical marker of HIV-disease severity, since most affected patients have CD4+ T-cells counts <400/mm³ (Araques et al,

Non-HIV-immunosuppressed patients in whom OHL can develop include patients who have undergone bone marrow (Epstein et al, 1988; Birek et al, 1989; Epstein et al, 1991; 1993b), renal (Itin et al, 1988; Greenspan et al, 1989b; Macleod et al, 1990; Kanitakis et al, 1991; King et al, 1994), hepatic (Reggiani and Pauluzzi, 1990; Schmidt-Westhausen et al, 1993) and cardiac (Schmidt-Westhausen et al, 1990; 1991) transplantation, those receiving chemotherapy for acute myeloid leukaemia (Syrjanen et al, 1989b) and multiple myeloma (Blomgren and Back, 1996), in patients administered long term corticosteroid therapy (Fluchiger et al, 1994; Lozada-Nur et al, 1994; Zakrzewska et al, 1995; Schiodt et al, 1995; Miranda and Lozada-Nur, 1996) and also in patients with myelodysplastic syndrome (Ficarra et al, 1991b) and Anderson-Fabry’s Disease (de Kaminsky et al, 1995). Further details of OHL in non-HIV-immunosuppressed patients are shown in Table 3:3. A few cases have been reported in immunocompetent individuals (Eisenberg et al, 1992; Felix et al, 1992; Lozada-Nur et al, 1994).

No cases of malignant transformation in pre-existing OHL have so far been reported (Greenspan and Greenspan, 1989). Mild cellular atypia has been described, in the form of slight disorganization of the basal cell and the
suprabasal prickle cell layers, hyperchromatism, pleomorphism of basal cells, and a small increase in the mitotic frequency (Greenspan et al, 1984). In this context, it may be worth noting that salivary IgA antibodies to EBV, detected primarily in patients with nasopharyngeal carcinoma (NPC), are also present in the saliva of OHL patients (Resnick et al, 1990), and sequences in the LMP-1 coding gene with deletions and nucleotide variations similar to the LMP-1 sequences in nasopharyngeal carcinoma and lymphoma tissues have been identified from cDNA recovered from OHL biopsies (Palefsky et al, 1996). Keratin phenotype profiling cast light on the premalignant state of OHL. However, the data have been conflicting. One group found reduced expression of keratin 19, which is the smallest of the keratins and one that is associated with premalignant change (Williams et al, 1991), but other groups were not able to confirm this (Thomas et al, 1991; Su et al, 1993).

Diagnosis

OHL needs to be distinguished from other white oral lesions. The differential diagnosis of OHL is listed in Table 3: 4. Since OHL almost always occurs in the context of immunosuppression, the clinician, upon detection of an OHL-like lesion, should seek to evaluate the immune status of the patient further. Generally, the laboratory findings, interpreted in conjunction with other signs of immune dysfunction and the social and medical history of the patient, should provide clues to the diagnosis and the underlying cause of immunosuppression. A provisional diagnosis of OHL can usually be based on clinical features alone
Histologic examination is indicated only when clinical features are vague. However, the koilocytotic changes are not specific to OHL (Eversole et al, 1986; Ficarra et al, 1991a) and even the normal lateral border of the tongue can yield a corrugated histological pattern with mild parakeratosis and keratin projections (Andersen et al, 1990; Brehmer-Andersson et al, 1994), particularly when the tongue tissue is sectioned incorrectly (Andersen et al, 1990). Furthermore, “pseudo-hairy” leukoplakia may need to be considered. The term applies to lesions that clinically and histologically resemble OHL, but upon further examination reveal no evidence for the presence of replicating EBV (Green et al, 1989b; McMillan et al, 1989; Fisher et al, 1992; Itin et al, 1993; Euvrard et al, 1994). Whether this is a distinct nosologic entity is unknown. Consequently, while an OHL-like lesion in an immunosuppressed individual whose histologic appearance resembles OHL can with confidence be regarded as OHL, this diagnosis should be considered presumptive. For definitive diagnosis further procedures are required.

The demonstration of replicating EBV is essential to the definitive diagnosis of OHL (Table 3:5). From biopsy sections, ISH using EBV-specific DNA probes is a reliable and sensitive procedure, that is adequate in most instances. The use of immunohistochemistry to detect EBV antigens in sections may appear straightforward, but the results can be variable; the choice of antibody panels to stain for the antigens plays an important role in producing reliable results.
Exfoliative cytology can make important contributions to diagnosis. As the cellular pathology of OHL extends across the thickness of the epithelium, diseased cells are easily removed by standard exfoliative cytologic techniques, following which, further characterization may be carried out (Lumerman et al., 1990; Fraga-Fernandez and Vicandi-Plaza, 1992). This approach is a useful alternative to incisional biopsy, for which there are often contra-indications (eg, bleeding disorders, children or severe debilitation). The observation of "nuclear beading" in the vacuolated or ballooned squames provides presumptive evidence for EBV replication.

More definitive procedures include ISH (DeSouza et al., 1990; Langford et al., 1992b; Migliorati et al., 1993) or ultrastructural examination for herpesviruses (Kratochvil et al., 1990; Sugihara et al., 1990b; Schmidt-Westhausen et al., 1990; 1991; 1993; Itin et al., 1994; Epstein et al., 1995) (Table 3:5). The negative staining EM method of Sugihara and co-workers, (1990), is simple and convenient, in that oral scrapings need only to be mixed with water, after which, they are examined for herpes-like particles; an Airfuge centrifugation step prior to EM enables the sensitivity of the procedure to be enhanced.

Cytological preparations may also be extracted following which, filter hybridization may be applied for EBV DNA detection (De Souza et al., 1990; Naher et al., 1991). The demonstration of EBV replication in specimens requires judicious use of laboratory tools. ISH should not be exquisitely sensitive. A sensitivity level set to a detection level of 100 genomes per cell (i.e, the genomic
content of Raji cells) is adequate. Polymerase chain reaction (PCR) assays for EBV DNA in extracts are not indicated in diagnostic contexts since salivary EBV, which are shed even in normal healthy people, will also be amplifiable, and low-level replication in the oral epithelium of HIV-infected people frequently occurs in the absence of overt OHL (Boulter et al, 1996).

Management

As OHL is usually asymptomatic (Reichart et al, 1989), may resolve spontaneously (Greenspan et al, 1984; Podzamczer et al, 1990; Katz et al, 1991), and has no known premalignant potential (Greenspan and Greenspan, 1989), treatment is seldom required.

Topical retinoids (eg. 0.1% vitamin A) are reported to lead to improvement in the appearance of OHL-affected oral surfaces (Schofer et al, 1987; Ochsendorf et al, 1988; Alessi et al, 1990), through their dekeratinizing and immunomodulating effects. But they are expensive, and when used for prolonged periods, lead to a burning sensation over the treated area (Lozada-Nur, 1991). Another modality is topical podophyllin, which has been reported to lead to short-lasting resolution of OHL (Lozada-Nur, 1991; Lozada-Nur and Costa, 1992; Sanchez et al, 1992; Gowdey et al, 1995b). Surgical excision (Herbst et al, 1989) and cryotherapy (Goh and Lau, 1994) have also been employed, but lesions can recur. Antifungal therapy may lead to some reduction in the extent of the lesion, but does not eradicate the infection (Greenspan et al, 1984; Ficarra et al, 1988b).
Antiviral agents such as acyclovir (Friedman-Kien, 1986; Schofer et al., 1987; Ficarra et al., 1988b; Resnick et al., 1988; Ochsendorf et al., 1988; Birek et al., 1989; Reichart et al., 1989; Herbst et al., 1989; Brockmeyer et al., 1989; Glick and Pliskin, 1990; Naher et al., 1990; Laskaris et al., 1995), zidovudine (Phelan and Klein, 1988; Kessler et al., 1988; Reichart et al., 1989; Brockmeyer et al., 1989; Katz et al., 1991), desciclovir (Greenspan et al., 1990), ganciclovir (Newman and Polk, 1987), foscarnet (Albrecht et al., 1994) and dideoxyinosine (ddI) (Cooley et al., 1990) can result in amelioration of overt OHL (Table 3-6), although lesions tend to recur soon after discontinuation. Nevertheless, systemic therapy with antiviral drugs is generally not indicated, given the side effects of antivirals and the documented resistance to antivirals after long term use. What the impact multiple antiretroviral therapy has on OHL, particularly when given early in HIV disease, will be watched with great interest.
CHAPTER 4

AIMS OF THE THESIS
AIMS OF THE THESIS

To examine various oral and dental aspects of HIV disease:

Specifically:

• to develop, a novel, non-isotopic, method, for detecting mutations

• to determine any possible inter and/ intra patient variation of Epstein-Barr virus (EBV) strains or genotypes in HIV-infected hosts, and the possible biological implications of such diversity.

• To assess the willingness of dentists in the United Kingdom (UK), to treat HIV-infected persons and assess the knowledge of UK dental students regarding HIV infection.

• to assess the prevalence of HIV among patients attending an out-patient clinic in a major inner-city Dental Hospital in UK.
CHAPTER 5

DIVERSITY OF EPSTEIN-BARR VIRUS CARRIED BY HIV-IMMUNOCOMPROMISED INDIVIDUALS: A MOLECULAR EPIDEMIOLOGIC STUDY
Introduction

Epstein-Barr virus (EBV), a gamma-herpes virus widespread in human populations, is increasingly recognised as being involved in a wide range of disease processes, many involving the head and neck (Gratama and Emberg, 1995). The virus is the cause of infectious mononucleosis (IM) (Henle et al., 1974), and is strongly associated with African Burkitt’s lymphoma (Zur Hausen et al., 1970), nasopharyngeal carcinoma (NPC) (Niedobitek et al., 1996), oral hairy leukoplakia (OHL) (Greenspan et al., 1985), Hodgkin’s disease (Weiss et al., 1991; Pallesen et al., 1991), as well as, with other cancers (Imai et al., 1994; Labrecque et al., 1995; Kotsianti et al., 1996).

The virus is tropic for epithelial cells (Sixbey et al., 1984; Greenspan et al., 1985) and B lymphocytes (Nilsson et al., 1971) in vivo. After primary infection, the virus establishes life-long latency in the host; this association of EBV with its hosts has given rise to a genetically diverse virus population. Two EBV types (designated as EBV-1 and 2 or type A and B) have been recognized. These types are essentially homologous across large stretches of the genome. The distinction between them stems from sequence divergence between the B95-8 (type 1) and AG876 (type 2) EBV genotypes within the open reading frame (ORF) encoding for the EBNA-2 antigen, which is located in the BamHI YH region of the genome (Dillner et al., 1985), and the open reading frames of EBNA-3A, 3B and 3C genes, tandemly arranged in the BamHI E region (Sample et al., 1990).
Type 1 strains are more prevalent in the Western, East Asian and North African populations, while the type 2 appears to be equally distributed in equatorial Africa, Papua New Guinea and Alaska (Abdel-Hamid et al., 1992). However, the epidemiologic picture is not clear-cut, as type 2 detection is also frequently found in Western patients who are immunosuppressed or have Hodgkin’s disease (Gratama and Emberg, 1995), and even in apparently healthy people (Sixbey et al., 1989; Apolloni and Sculley, 1994).

In most instances, the genetic changes in the EBV genome are not functionally manifested, as most alterations involve non-coding regions of the viral genome. However, there are biological differences between the two types in vitro, in that, type 1 grow out to lymphoid cell lines more quickly than type 2, and show slightly lower levels of spontaneous entry in lytic cycle (Rickinson et al., 1987).

More recently, a number of other genomic polymorphisms which can help to identify individual strains have been noted, but in the latter cases, there are some disease-associations. EBV variants can be distinguished according to the retention or loss of an Xho I restriction site in the LMP-1 gene, or the presence or absence of a 30-base pair (bp) deletion at the 3’ end of the LMP-1 ORF (Hu et al., 1991). Variants without the Xho I restriction site and possessing the 30-bp deletion are positively correlated with nasopharyngeal carcinoma (NPC) and lymphoproliferative disorders (Jenkins and Farell, 1996).
Further differentiation of EBV variants can be based on a BamHI restriction site between the BamHI W1* and I1* fragments, and an additional BamHI site in the Bam H1 fragment, giving rise to the C/D and F/f genotypes, respectively (Lung et al., 1990); these genotypes may be geographically restricted (Gratama and Ernberg, 1995). More recently, distinct intra-host EBV genotypes containing either two or three copies of a 29-bp tandem repeat sequence in the first intron of the BZLF1 gene have been reported (Chen et al., 1996), with the genotype that bears 3 copies appearing to infect the epithelium preferentially. In addition, EBV variants which escape cytotoxic T-cell recognition are common among isolates from Papua New Guinea and China (de Campos-Lima et al., 1994).

Other than these dichotomies, EBV can be differentiated according to size polymorphisms, depending on the number of internal repeats in the BamHI E, H, I, K and N regions (Lung et al., 1988; Miller et al., 1994; Falk et al., 1995). A variety of natural EBV variants have been discovered, and inter-person transmissions traced (Lung et al., 1991; Cen et al., 1991; Alfieri et al., 1996; Falk et al., 1997). Furthermore, discrete mutations involving point substitutions, and deletions or insertions of single bases or longer stretches in other genomic regions, have also been observed. These would potentially allow variants to be even more finely discriminated. Hypervariability has thus been identified in: the C-terminal domain of EBNA-1 ORF in the BamHI K region (Wrightham et al., 1995; Snudden et al., 1995; Bhatia et al., 1996); the EBNA-2 gene, which is located in the BamHI WYH region (Aitken et al., 1994); several domains in the BamHI N region, including the LMP-1 gene (Miller et al., 1994), the N-terminal
of the LMP-2A gene (Busson et al, 1995), and an approximately 300-bp stretch upstream of the LMP-1 start codon (Hu et al, 1991), and the BZLF-1 gene (Packham et al, 1993).

In this chapter, sequence polymorphisms have been investigated, using DNA sequencing and single-strand conformation polymorphism (SSCP) assays, in potential hypervariable domains of the BamHI K, N and Z regions in EBV-carrying cell lines. Possible hypervariability in the type 1/2-specifying locus within the BamHI E region encoding EBNA 3C (Sample et al, 1990) has also been examined. The same SSCP procedure has then been applied to investigate genetic diversity of EBV carried in various tissue sites and body fluids of hosts who were and were not co-infected by human immunodeficiency virus (HIV).

Materials and Methods

Cell lines.

The EBV-infected lymphoid cell lines B95-8, EB176, EB185, P3HR-1, AW-Ramos, AG876, Namalwa, Daudi and Raji (all obtained from the European Collection of Cell Cultures, Porton Down, England) were propagated in RPMI 1640 supplemented with 10% bovine calf serum. The EB176 and 185 lines are derived from peripheral lymphocytes of a chimpanzee and an orangutang, respectively, having been transformed by virus of the B95-8 line. EBV carried by these and the Namalwa, Daudi and Raji cell lines are type 1 viruses. AW-Ramos is a converted cell line, being derived from the Ramos cell line that was infected
by EBV from P3HR-1 cells (Andersson and Lindhal, 1976). EBV in the AW Ramos, P3HR-1 and AG876 lines are type 2 viruses.

Clinical and biopsy specimens.

Tongue scrapings were obtained from 20 serologically-proven HIV-infected outpatients of the Infectious and Parasitic Diseases Service of the Hospital das Clinicas, Universidade Federal de Pernambuco, Recife, Brazil, by scraping the lateral border of the tongue with a sterile curette, washed in phosphate buffered saline and pelleted (Boulter et al., 1996). From the same cohort of patients, peripheral blood cells (PBCs) were drawn into EDTA-treated vacutainers, and enriched for CD45+ cells by using an immunomagnetic bead procedure, performed as recommended by the manufacturer (Dynal, Oslo, Norway).

Whole mouth and parotid saliva, were obtained from 3 serologically-proven, HIV-infected outpatients of the Department of Primary Dental Care, King’s College, School of Medicine and Dentistry. Whole saliva was collected after mechanical stimulation from chewing sterile rubber bands, clarified and passed through 5.0 μm Acrodisc filters. To collect parotid saliva, a Lashley cup was applied to Stensen’s duct following stimulation of the tongue with citric acid crystals, after which salivary fluid was passed through 0.45 μm filters (Ochert et al., 1994).
Tissue biopsy specimens were derived from the following: 2 with diagnosis of bronchogenic carcinoma, 2 squamous cell carcinomas of the skin, 1 oral ulcer, 1 oral hairy leukoplakia (OHL), 1 oral mucosa from a patient with Sweet’s syndrome and 1 NPC; all were formalin-fixed and paraffin-embedded. The specimens were provided by the Department of Oral Medicine and Pathology, University of Chieti, Italy.

**DNA extraction.**

DNA was extracted from cells and saliva specimens using the Chelex-100 extraction protocol (Ochert et al, 1994), from tongue samples by the proteinase K digestion method, and from blood samples and tissue sections using Geneclean II (BIO 101 Inc, Vista, California).

**PCR amplification.**

Primers were selected to allow amplification: by nested PCR, a 433-bp segment in the EBNA-1-coding domain of the BamHI K region (Wrightham et al, 1995); by heminested PCR, a 386-bp segment that encompasses and is upstream of the LMP-1 start codon in the BamHI N region (Hu et al, 1991); by single-round PCR, a 394-bp segment in the first intron of the BZLF-1 gene (Packham et al, 1993); and by single-round PCR, a 246-bp segment bracketing the type 1/2-specifying domain of the EBNA-3C gene in the BamHI E region (Sample et al, 1990). Sequences and coordinates of the primers are shown in Table 5:1. The
EBNA 3C-coding region was examined even though sequence polymorphism therein may not be as marked as that in the BamHI K, N and Z regions, because of its potential to discriminate reliably between types 1 and 2 (Sample et al., 1990).

PCR was performed according to standard procedures. The reaction mixture (50 μl) consisted of 20 pM of each primer, 50 mM MgCl₂, 5 units Taq DNA polymerase and 100 mM each of the deoxynucleoside triphosphates. PCR was performed for 35 cycles (denaturation at 94°C for 1 min, annealing at 60°C (68°C for the K primers) for 40 sec and extension at 72°C for 1 min). Before the start of each reaction, the samples were denatured at 94°C for 5 min; after the last cycle, the polymerization step was extended by 10 min. Further amplification of BamHI K and N amplicons was carried out, as follows: 2 μl of the first-round PCR reaction was added to 48 μl of the second-round PCR mix, which contained the inner primers of BamHI K amplicon and the heminested anti-sense primer of the BamHI N region. The mixtures underwent 25 cycles amplification for the BamHI K primers (1 min at 94°C, 40 sec at 60°C, 1 min at 72°C) and 35 cycles amplification for the BamHI N primers (1 min at 94°C, 40 sec at 62°C, 2 min at 72°C).

**PCR sequencing**

Initial experiments examined the extent of DNA sequence heterogeneity in the EBV-infected cell lines by cycle-sequencing. PCR products were purified using
Geneclean II, and sequenced using the Taq DyeDeoxy Terminator Cycle Sequencing Kit and the ABI 373A DNA sequencer (Perkin-Elmer, Forest City, California). Routinely, 2 opposing strands from each product were sequenced. Inter-primer base sequences were aligned and compared using the Clustal algorithm in the MEGALIGN program of the LASERGENE System (DNASTAR Inc, Madison, Wisconsin).

**Single-strand conformation polymorphism (SSCP) assay**

PCR-based single strand conformation polymorphism analysis (SSCP), is the most widely used indirect method for mutation detection, but requires radioactive probes, difficult to handle gels, lengthy electrophoresis etc. Some non-isotopic detection procedures have been developed (eg. staining with silver or ethidium bromide), which have their own limitations.

The present study attempted to assess a new method for detecting mutations, using a novel nucleic acid stain, SYBR-GREEN II (FMC BioProducts).

Initial experiments used PCR fragments, amplified from domains of the Hepatitis B virus (HBV) genome, encoding for the surface and core antigens. The PCR products were run (at different dilutions), in a horizontal high resolution agarose gel (Metaphor), containing (or not) glycerol (at different concentrations), both at room temperature and 4°C, at different voltage, and different running time. The gels were then stained for comparison, both with ethidium bromide and SYBR-
GREEN II, and photographed. In all experiments, very faint bands were observed (stained by both ways), unsuitable for reliable interpretation.

As an alternative to the Metaphor gel, a high resolution polyacrylamide gel MDE (Mutation Detection Enhancement) gel (FMC BioProducts, Rockland, USA) in a vertical mini-electrophoresis Hoefer 600 apparatus was applied. The MDE gel is a polyacrylamide-derived matrix, which has been optimized for resolving conformational differences and has been shown to improve the sensitivity of SSCP analysis. Using the same PCR products, (from the HBV genes), clearly visible bands were detected, when the gel was stained by SYBR-GREEN II.

Finally, the following procedure was developed to assess sequence changes in EBV subgenomic amplicons amplified from cell lines and clinical specimens. Ten µl of each original product was mixed with 10 µl of stop solution (95% formamide, 10 mM NaOH, 0.05% bromophenol blue and 0.05% xylene cyanol), heat-denatured at 95°C for 5 minutes, cooled on ice and applied immediately to 0.5X MDE gel. SSCP gels were run at constant voltage (280 V) and temperature (4°C). The optimal running times were determined empirically (after sequential experiments) for each genome region (24 hr for BamHI K, 15 hr for BamHI N and BamHI Z, and 5 hr for BamHI E). The gel was then stained with SYBR-GREEN II according to manufacturer’s instructions, and photographed. The SSCP procedure was subsequently applied to EBV DNA fragments amplified from clinical and tissue specimens.
SSCP procedure was subsequently applied to EBV DNA fragments amplified from clinical and tissue specimens.

**PCR cloning**

Clones were generated from the PCR products derived from the OHL and NPC tissues using the LigATor cloning kit (R & D Systems, Minneapolis, Minnesota). Following transformation into competent *Escherichia coli* cells, 16 white colonies were picked for analysis.

**Results**

**EBV subgenomic diversity in cell lines.**

The pattern and extent of DNA sequence polymorphism differed in the 4 subgenomic regions (Fig. 5:1). In the amplified *BamHI* E segment, type 1- and 2-specifying motifs were readily evident, particularly the 93-bp insertion in type 2. Between types, the degree of variation was low: compared to B95-8, there were 1 and 4 base changes in Namalwa and Daudi (0.9 and 3.6% dissimilarity), respectively, while P3HR-1 differed from AG876 by 1 base (Fig.5:1A). Within the *BamHI* Z fragment, type 1 and 2 differences were also apparent, particularly the 29-bp insertion in the type 2 variants. Again, the extent of variation was small between types: Namalwa and Daudi shared the same sequence, differing from B95-8 by one base substitution, while there were 5 base changes between P3HR-
1 and AG876 (1.5% dissimilarity) (Fig. 5:1B). DNA fragments from the BamHI E and Z regions were unamplifiable from Raji cells, reflecting possible deletions in these regions (Rymo et al, 1981). In the fragments amplified from the BamHI N and K regions, no type-specific differences could be discerned, but there were variations between lines. For the BamHI N segment, dissimilarities from B95-8 ranged from 2.3% in AG876 to 7.2% in P3HR-1 and Namalwa (Fig. 5:1C), while for the K segment, dissimilarities from B95-8 ranged from 4.1% in P3HR-1 to 5.9% in Namalwa (Fig. 5:1D).

Following agarose gel electrophoresis of DNA fragments amplified from the 4 EBV subgenomic regions, only type-specific size polymorphisms were evident (Fig 5:2A-D, upper panels).

When the PCR products were subjected to SSCP analysis (Figure 5:2A-D, lower panels), intra-type mobility shifts were not observed with the BamHI E PCR products (Fig 5:2A, lower panel), but were evident between P3HR-1/AW Ramos and AG876 for the BamHI Z fragment (Fig 5:2B, lower panel), and between all the N and K products known to bear unique sequences (Fig. 5:2C and D, lower panels). The band positions of the BamHI N and K amplicons between B95-8, EB176 and EB185, and between P3HR-1 and AW Ramos, were indistinguishable (Fig. 5:2C and D, lower panels) (for the N fragment there were variations in intensity of the lower bands; Fig. 5:2C, lower panel).
EBV BamHI N and K sequence diversity in clinical specimens.

The data from the cell lines showing that the BamHI N and K segments were more hypervariable than those of E and Z segments (Figs. 5:1 and 5:2) led us to investigate whether naturally occurring EBV may also exhibit sequence polymorphisms in the N and K regions. To avoid sequencing all the N and K amplicons, we used SSCP to scan for nucleotide differences. When applied to EBV-positive tongue scrapings of HIV-infected people, differences in SSCP banding patterns between each patient were evident (Fig. 5:3A). When SSCP assay was applied to 10 HIV-infected patients from whom BamHI N amplicons could be obtained from both tongue scrapings and CD45-enriched PBCs (Fig. 5:3B), within-person identity of banding patterns was unequivocal in one patient (no. 10), and possible in four (nos. 2, 5, 7 and 8); for the rest, within-person differences were clearly apparent.

To examine if such intra-host differences apply to other body sites, the same procedure was used to examine BamHI N amplicons derived from whole saliva, tongue and parotid saliva of another HIV-infected patient (no. 11) in whom EBV DNA could be amplified from all three compartments; as Fig. 5:3C (left panel) shows, the patterns were distinguishable from each other. The procedure was further used to investigate if, over a period of time, an EBV variant shed into the oral cavity might change. As Fig. 5:3C (right panel) shows for patient 12 for whom, whole saliva was obtainable regularly over a 12-month period, the same pattern of mobility shift was seen at each sampling. The figure also shows that,
over the same period, the band positions from the saliva of patient 13, who was a homosexual partner of patient 12, was identical to patient 12 (there were some variations in banding intensities).

**EBV BamHI K sequence diversity in lesional specimens.**

When the SSCP procedure was applied to scan for sequence polymorphism in the BamHI K amplicons recovered from various lesions previously identified to be EBV-positive, inter-lesional variability was observed (Fig. 5:4). To examine, further, if different variants could be harboured by a given lesion, clones from BamHI K amplicons were derived from the OHL and the NPC specimen, and subjected to the SSCP assay. Fig. 5:5 shows that, while at least 3 SSCP banding patterns were discernable in the OHL lesion, all the bands of the NPC specimen were identical.

**Discussion**

The analysis of genetic polymorphism among EBV isolates has provided useful information regarding EBV distribution, transmission or carriage patterns and associations between EBV and certain diseases. While the distinction between type A and type B allows the division of EBV isolates into two broad categories, discrimination between EBV isolates on the basis of deletions of DNA, repetitive sequences within the genome, and/or the presence or absence of restriction sites allow further resolution.
Since EBV cannot be directly propagated in vitro, in order to investigate the diversity of naturally occurring EBV, the conventional approach has been to transform B cells into permanent lymphoblastoid cell lines, after which viral DNA or protein extract is submitted to various differentiation procedures, such as, DNA restriction-fragment length polymorphism (RFLP) assays (Lung et al, 1988), or Western blot analysis of EBNA size polymorphisms (“Ebnotyping”) (Gratama et al, 1994). A limitation to this approach is that, there is potential selection against EBV strains that either transform lymphocytes poorly, such as the type B viruses (Rickinson et al, 1987), or not at all, such as, EBNA-2-deletion variants (Sixbey et al, 1991).

Analysis by restriction fragment-length polymorphism (RFLP) in the two small RNA-encoding regions of EBV has demonstrated type-specific cleavage patterns between type A and B (Arrand et al, 1989). This method requires restriction endonuclease digestion of DNA, followed by Southern blot hybridization, a tedious and time consuming procedure.

Alternatively, EBV DNA sequences are amplified directly from a given tissue or body fluid, using procedures such as type-specific PCR (Buisson et al, 1994; Aitken et al, 1994; Menin et al, 1996), post-PCR type-specific oligonucleotide probing (Sixbey et al, 1989, Yao et al, 1991, Apolloni and Sculley, 1994), gel-electrophoretic analyses for size polymorphisms of PCR products (Lin et al, 1993; Miller et al, 1994; Falk et al, 1995, Chen et al 1996) and post-PCR RFLP assays (Bhatia et al, 1996). The discriminatory ranges of these procedures are
finite, so sequencing is often required to examine further heterogeneity within the amplified fragments (Buisson et al., 1994, Aitken et al., 1994, Miller et al., 1994; Sandvej et al., 1994; Busson et al., 1995; Bhatia et al., 1996). Obviously, the selection bias inherent in lymphocyte culture is avoided in this way, but heterogeneity in the coding regions of interest amongst wild-type EBV strains may prevent PCR primer recognition and lead to false negative results.

Another approach is PCR-based single strand conformation polymorphism analysis (SSCP), which has become the most widely used indirect method for mutation detection, mainly because of its sensitivity and simplicity (Orita et al., 1989a). This method has been demonstrated to be effective in detecting mutated genes in human cancers (Hensel et al., 1991), and in identifying disease-causing mutations (Dork et al., 1994). In the conventional SSCP analysis, radiolabelled PCR products are denatured to produce single-stranded (s.s) DNAs and electrophoresed through a nondenaturating gel (Orita et al., 1989b). Polymorphic differences in strand mobility result from the effects of primary sequence changes on the folded structure of a single strand. Mobility shifts observed between samples of the same fragment are indicative of alterations in DNA sequence between the samples.

However, conventional SSCP analysis requires radioactive hybridization probes and large, difficult to handle polyacrylamide gels, which often involve lengthy electrophoresis and extended autoradiography, and disposal of sizable volumes of radioactive waste. So, some non-isotopic detection procedures have been
developed, such as, staining with silver (Bosari et al, 1995), or ethidium bromide (Ballhausen and Kraus, 1993); fluorescence (Iwahana et al, 1994) and chemiluminescence detection (El-Aleem et al, 1995). Nevertheless, even the non-isotopic procedures have their own limitations: fragility of the gels; brown or yellow background which reduces the sensitivity; inability to use DNA bands as templates for further amplification; expensive apparatus for fluorescence detection etc. (Calvert et al, 1995; Binder et al, 1995).

In a recent study, Law and co-workers (1996), using SYBR-GREEN II stain in conjunction with the use of 20% non-denaturating polyacrylamide minigels and alkali denaturation, achieved rapid detection of single base alterations in DNA fragments up to 500-bp. Mobility shifts were clearly visible, when only 5% of the mutant DNA was present in the PCR template, when stained with SYBR-GREEN II, while mutant bands were not visible with ethidium bromide staining, until the sample contained 30% mutant DNA.

In the present study of EBV-infected cell lines, SYBR-GREEN II was shown to be effective in differentiating between different strains of EBV, but not as effectively, in all the segments of the EBV genome. This variability in the sensitivity of the SSCP analysis stain was not surprising. The sensitivity of SSCP for detecting mutations has been reported, as ranging from 35% to nearly 100% (Orita et al, 1989a; Orita et al, 1989b; Sarkar et al, 1992; Sheffield et al, 1993) with the highest detection rates most often requiring several different conditions. The factor that has the greatest effect on SSCP sensitivity is the size of the DNA
fragment. As the DNA fragment increases in length, the sensitivity of the SSCP analysis decreases. An optimal size of 200-bp or less is the most sensitive for single base substitutions. In a DNA fragment of 200-400 bp, the sensitivity of SSCP analysis decreases from 95-100% to 65-75%, while the minimum fragment has not been fully addressed in the literature.

Surprisingly, in this study, the PCR products of the lower size (BamHI E region) gave the poorer results, while the best resolution was achieved with the 386-bp fragment in the BamHI N region, and the 433-bp fragment in the BamHI K region. This can be explained by the fact that, the BamHI N and K primers were designed to encompass highly heterogenic areas of the EBV genome (Fig. 5:1).

Detailed sequencing studies have identified variability in the BamHI N region (Hu et al, 1991), and, in particular, the heterogeneity of the LMP-1 ORF has been verified in a broad range of EBV-associated lesions (Miller et al, 1994; Sandvej et al, 1994; Palefsky et al, 1996). Other reports have alluded to sequence heterogeneity in the C-terminal domain of the EBNA-1 gene (Wrightham 1995; Snudden et al, 1995, Bhaita et al, 1996). In contrast, the results of this study, indicates that, the segment amplified from the EBNA-3C gene (Fig. 5:1 and 5:2A) will not be useful as a polymorphic marker in wild type EBV other than for discriminating between types 1 and 2.

The PCR-SSCP analysis, although sensitive, has its inherent limitations. For instance, a particular point mutation might not be detected, if it occurred in a loop
or in a long stable stem of the secondary structure. Thus, single-base changes that do not alter the conformation of the single strands would remain undetectable by SSCP. Without doubt, the present approach is recommended, as it further simplifies SSCP analysis by eliminating the use of radioactivity, using easy-to-handle and cost-effective material, with clear post-staining background, lengthy electrophoresis, and of course without the risk of using hazardous chemicals.

Subsequently, this study was able to demonstrate, that the hypervariability in these regions is sufficiently wide to be used as an index of EBV diversity in body fluids and tissue specimens. This does not require DNA sequencing, as the SSCP procedure provides a satisfactory alternative for evaluating sequence polymorphisms in the 2 regions (Figs. 5:3 and 5:4). The non-isotopic nature of the SSCP approach, as described, potentially allows large numbers of EBV-infected tissues and body fluids to be screened for EBV genomic variation. Furthermore, the procedure provides a simple means of examining EBV clonality; the findings in Fig. 5:5 confirm the monoclonality of EBV in NPC, but not in OHL (Miller et al., 1994). As, there is sufficient sequence variability in the amplified BamHI N and K fragments to permit the use of the SSCP procedure to scan for EBV heterogeneity in body specimens, we test wild-type EBVs derived from a group of HIV-infected patients.

There is a substantial amount of evidence, regarding the interaction between EBV and HIV in the context of HIV infection. EBV can be isolated from the throat washings of 70-80% of HIV-infected homosexual (Crawford et al., 1984),
a degree of EBV excretion that is observed only in the setting of acute infectious mononucleosis; HIV carriers show higher titers of EBV in mouthwashes and in saliva (Lucht et al., 1995), as compared to healthy controls; the titers of EBV-related antibodies and the numbers of EBV-infected cells in the peripheral blood of HIV-infected individuals are much higher than those in normal healthy subjects (Rinaldo et al., 1986); oropharyngeal shedding of EBV may be a marker of risk for seroconversion to HIV (Ferbas et al., 1992), while, EBV infection has been implicated in AIDS-associated conditions, mainly in oral hairy leukoplakia (Greenspan et al., 1985).

Furthermore, direct interaction between EBV and HIV has been implied by experiments demonstrating that, EBV could either enhance HIV infection directly, by transactivation of HIV DNA (Mallon et al., 1990), or indirectly, by its own immunosuppressive properties (Rickinson et al., 1985), or by induction of cytokines that activate HIV DNA replication (Clouse et al., 1989). In addition, HIV can enhance EBV DNA replication in B lymphocytes (Lai et al., 1989), which, in turn, could contribute to disease progression, if EBV replication results in B-cell destruction.

Evidence suggests that co-infection with EBV strains of different type can occur, but is relatively rare in healthy individuals. Likewise, the co-residence of different viruses of the same type must also be rare, at least from the analysis of Caucasian populations. Thus, when multiple independent isolates have been made from individual donors, any one person appears to carry a single
characteristic EBV strain (usually type 1), which is dominant both in the blood and in the throat and which persists over time (Yao et al, 1991). By contrast, many authors have shown that co-infection may be more common in HIV-infected patients: Sixbey and co-workers (1989) for throat washings, Sculley and co-workers (1990) for LCLs and Kyaw and co-workers (1992) for PBMCs and LCLs.

Moreover, Walling and co-workers, (1992) observed that both types 1 and 2 could be present within an oral hairy leukoplakia lesion. Analysis of EBV types in cardiac transplant patients (Kyaw et al, 1992) makes the possibility that HIV per se is responsible for this EBV variability, unlikely.

In a detailed work, which has been carried out on a group of European patients receiving immunosuppressive therapy following bone marrow or heart transplantation (Gratama et al, 1994), three patterns of simultaneous presence of EBV strains (Ebnotypes) were distinguished by immunoblotting:

The first, most frequent pattern observed predominantly in oropharyngeal cultures consisted of minority Ebnotypes differing from the majority type by only a single EBNA protein (usually EBNA-1). The second, less frequent, pattern observed in the healthy carriers and the transplant recipients consisted of minority Ebnotypes differing from the majority type by two EBNA proteins (mostly EBNAs 1 and 6). The third pattern, characterized by the simultaneous presence of totally different Ebnotypes, was restricted to the transplant
recipients, and AIDS patients and was more frequently observed in the blood than in the oropharynx. The authors suggest that, the first two patterns result from heterologous recombinations occurring during viral replication at repeat sequences within the EBNA coding regions, whereas the third pattern reflects multiple infections with exogenous viruses (Gratama et al, 1994).

The present study demonstrated intra-host diversity in proximate oral compartments (Fig. 5:3C, left panel), suggesting that different variants can occupy specific niches. Whether, such occupation reflects selective tropism of EBV is unknown. Identification of the same EBV variant from the mouths of patients 11 and 12 over a protracted period (Fig. 5:3C, right panel) may be due to that variant being persistently shed into their oral cavities from unidentified sites or to persistent reinfection from one partner to the other.

EBV carrier state in AIDS patients may be similar to that which exists in the general population, but that the true spectrum of resident viruses is easier to see in such patients, because of their higher viral load. This view would imply that many healthy individuals are also infected with multiple viral strains, but that is gone undetected in conventional screening assays.

An alternative view is that the multiple EBV infections seen in many AIDS patients, are an opportunistic consequence of immune impairment and do not reflect the situation in healthy carriers. One explanation for the co-existence of multiple EBV strains within a single individual would be superinfection with
exogenous strains, either via cell-borne virus (through blood transfusions, bone marrow or organ allografts) or via cell-free virions (through salivary contacts). Alternatively, EBV strain diversity may be generated by recombination between linear DNA molecules during virus replication. Indeed, Yao and co-workers, (1996a) provided, in HIV-infected individuals, the first evidence for the existence in the wild of intertypic EBV recombinants, which retain both transforming and replicative functions. Since HIV-immunocompromised patients show greater susceptibility to multiple EBV infections, and elevated levels of EBV replication occurring at permissive sites in these individuals (Greenspan et al, 1985), the chances of a permissive target cell, of either epithelial or B-cell origin, being co-infected with both virus types are high; this is particularly so because type 1 and type 2 viruses appear to show the same tropism, both for B lymphocytes and for oropharyngeal epithelial cell in vivo, and might therefore, establish co-infections at the cellular level.

The present study has therefore shown that, variation in certain genomic segments of naturally occurring EBV can be extensive, extending beyond conventional groupings based on dichotomous genotypic differences and the number of internal nucleotide repeats. What mechanisms might account for such microheterogeneity? Immune pressure exerted by cytotoxic T-lymphocytes (CTLs) is a possible factor. Sequence polymorphisms potentially affecting the recognition of MHC Class I and 2-restricted CTL epitopes, predominantly in EBNAs 2, 3A, 3B and 3C, and LMPs 1 and 2A, have been reported (Khanna et al, 1995; Burrows et al, 1996). Indeed, the DNA fragment amplified from the
BamHI K region in this study has been identified to encompass potential Class I-restricted epitopes in EBNA-1 (Wrightham et al., 1995). (The hypervariable segments encoding known CTL epitopes in the BamHI E and N regions are distinct from the ones used in the current study.) Frequent mutations in these epitope-specifying regions are likely to aid viral immune evasion by preventing CTLs from recognizing proteins expressed in infected cells.

While CTL-driven diversity might account for inter-population or inter-ethnic differences in the EBV genome, it is more difficult to explain how this can generate inter- or intra-person viral variation to the extent demonstrated in this study. We have already noted that the hypervariable BamHI N fragment examined here is not known to encode epitope-specifying segments (Hu et al., 1991). One mechanism of generating intra-person diversity might be homologous genetic recombination between co-infecting variants undergoing high levels of vegetative replication. Thus, recombinants have been demonstrated in the peripheral blood and throat of HIV-infected hosts (Yao et al., 1996b), and in OHL lesions of people regardless of their HIV status (Walling et al., 1992; 1994; 1995). Nevertheless, homologous recombination does not fully account for inter-person EBV variation, particularly among people who are not HIV-infected (Fig. 5:4).

Given the relative immutability of herpes viruses (Smith and Inglis, 1987), the existence of hypermutable loci in EBV is unexpected, and further studies into how genetic microheterogeneity support the survival of this virus should be rewarding.

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CHAPTER 6

TISSUE DISTRIBUTION OF EPSTEIN-BARR VIRUS GENOTYPES IN HOSTS CO-INFECTED BY HUMAN IMMUNODEFICIENCY VIRUS: IMPLICATIONS FOR THE PATHOGENESIS OF ORAL HAIRY LEUKOPLAKIA
Introduction

Oral hairy leukoplakia (OHL), a lesion typically appearing as a white patch on the lateral margin of the tongue, is an early clinical marker of HIV infection and is associated with increased severity of HIV disease (as detailed in Chapter 3). Epstein-Barr virus (EBV) undergoes productive infection in keratinocytes affected by OHL. How EBV enters the lingual epithelium of the HIV-coinfected host, where the infecting EBV originates, and to what extent EBV contributes to the pathogenesis of OHL, are unknown.

Epstein-Barr virus (EBV) is genetically diverse (Gratama and Ernberg, 1995). Two EBV genotypes (designated as EBV-1 and 2, or type A and B) have been recognized on the basis of sequence divergence in the open reading frames encoding the EBNA-2, EBNA-3A, 3B and 3C antigens (Sample et al., 1990; Gratama and Ernberg, 1995). Lymphocyte cell lines transformed by type B, compared to type A viruses, grow out more slowly and are difficult to expand into long-term growing cell lines (Rickinson and Kieff, 1996); thus, the classification of EBV into types A and B may have a biological basis.

A number of other polymorphisms in the EBV genome has also been observed. These are based on: the presence or absence of a 30-base pair (bp) deletion at the 3' end of the LMP-1 gene; the retention or loss of an Xho I restriction site in the LMP-1 gene; a Bam HI restriction site between Bam HI W1* and I1* fragments and an additional Bam HI site in the Bam HI F fragment (Hu et al., 1991; Gratama
and Emberg, 1995). In contrast to the A/B polymorphism, the functional significance of these latter polymorphisms is less clear (Gratama and Emberg, 1995).

Different EBV variants may preferentially infect specific tissues. In the healthy host, EBV is tropic for the epithelium and B-lymphocytes (Rickinson and Kieff, 1996). It has been reported, that variants bearing three copies of a 29-bp tandem repeat sequence in the first intron of the BZLF-1 gene are predominantly found in the epithelium, while those with two copies are more frequently detected in peripheral blood (Chen et al, 1996). This chapter has investigated, in hosts co-infected by HIV-1, whether EBV variants grouped according to the polymorphism in the BZLF-1 gene, show changes in their tissue tropism, and evaluated if this polymorphism segregates with the type A and B polymorphism, and the 30-bp polymorphism in the LMP-1 gene.

**Materials and Methods**

**Clinical and biopsy specimens**

Clinical specimens were obtained from 20 serologically-proven HIV-infected outpatients of the Infectious and Parasitic Diseases Service of the Hospital das Clinicas, Universidade Federal de Pernambuco, Recife, Brazil. None showed clinical evidence of oral hairy leukoplakia (OHL), according to established criteria (Triantos et al, 1997). As controls, 40 healthy laboratory staff volunteers
were recruited; these were not tested for their HIV status and were assumed to be HIV-negative. Local ethical approval was given to the study and informed consent was obtained from all the participants.

Tongue scrapings were obtained by scraping the lateral borders of the tongue with a sterile curette, washed in phosphate buffered saline and pelletted (Boulter et al, 1996). Peripheral blood cells (PBCs) drawn into edetic acid-treated vacutainers were enriched for CD45+ cells by using an immunomagnetic bead procedure, performed as recommended by the manufacturer (Dynal, Oslo, Norway).

**DNA extraction**

DNA was extracted from tongue specimens by the proteinase K digestion method, and from blood samples using Geneclean II (BIO 101 Inc, Vista, California). As further controls, DNA extracted from the cell line carrying prototypic type A EBV, AG876 and the line carrying prototypic type B EBV, B95-8, was used (see Chapter 5).

**PCR amplification**

Extracts underwent PCR to amplify a 110-bp segment of the beta-globin; all were determined to carry this segment. To amplify DNA from the BZLF-1 region, we used primers flanking a 374-bp segment in the first intron of the gene
(coordinates 102,304-102,669) (Packham et al., 1993). This heminested primer set was found to reproducibly yield specific products after an extensive evaluation of other sets, including primers pairs that nested within the outer primers. For the LMP-1 encoding gene, the primers flanked a 262-bp segment in exon C of the BNLF-1 gene (coordinates 168,096-168,401) (Chen et al., 1996). For the EBNA-3C encoding gene, the primers flanked a 246-bp segment bracketing the type A/B-specifying domain in the Bam HI E region (coordinates, 99,939-100,091) (Sample et al., 1990). Sequences of the primers are shown in Table 6:1.

PCR was performed according to standard procedures. The reaction mixture (50 μl) consisted of 20 pM of each primer, 50 mM MgCl₂, 5 units Taq DNA polymerase and 100 mM each of the deoxynucleoside triphosphates. PCR was performed for 35 cycles (denaturation at 94°C for 1 min, annealing at 60°C for 40 sec and extension at 72°C for 1 min). Before the start of each reaction, the samples were denatured for 5 min at 94°C; after the last cycle, the polymerization step was extended by 10 min. Further amplification of amplicons in the BZLF-1 and LMP-1 gene was carried out by applying 2 μl of the first-round PCR reaction to 48 μl of the second-round PCR mix and submitting the mixture to 35 cycles of PCR under the same conditions as the first round.

PCR cloning
Clones were generated from the PCR products derived from epithelial and PBCs of patients 1 and 8, using the LigATor cloning kit (R & D Systems, Minneapolis, Minnesota). Following transformation into competent *Escherichia coli* cells, white colonies were picked for analysis.

**Single-strand conformation polymorphism (SSCP) assay**

A non-isotopic SSCP procedure was used to assess sequence changes in EBV amplicons derived from clones. In brief, 10 µl of each original product was mixed with 10 µl of stop solution (95% formamide, 10 mM NaOH, 0.05% bromophenol blue and 0.05% xylene cyanol), heat-denatured at 95°C for 5 min, cooled on ice and applied immediately to 0.5X MDE gel (PMC BioProducts, Rockland, USA) in a vertical mini-electrophoresis Hoefer 600 apparatus. SSCP gels were run at constant voltage (280 V) and temperature (4°C) for 15 hr. The gel was then stained with SYBR-GREEN II (FMC BioProducts) according to manufacturer's instructions, and photographed.

**Results**

**Z₂ and Z₃ genotyping**

The first intron of the BZLF1 gene of EBV from AG876 cells contains three copies of a tandem repeat sequence, one more copy of the repeat sequence than that of the B95-8 virus. The genotype of AG876-like viruses, designated Z₃,
yields a 374-bp product, and can be distinguished from the shorter, 346-bp product derived from B95-8-like viruses, designated Z_2(Fig. 6:1).

In the HIV-negative group, the Z_2 genotype was detected in all PBCs of 7 patients from whom products were amplifiable from the BZLF-1 gene (Fig. 6:1). In four other HIV-negative patients, the tongue specimens of three (patient nos. 32, 39 and 40) were positive for the Z_3 genotype, and the specimen of one patient (no. 28) was positive for Z_2 (Fig. 6:1). The difference in the proportion of Z_2 and Z_3 genotypes in the tongue and blood of this group was significant (p=0.024; Fisher's exact test, 2-tailed). In the HIV-infected group, the Z_2 genotype was detected in PBCs of 11 of 14 patients (nos. 1, 3, 4, 7, 8, 12, 14, 16, 17, 18 and 19), Z_3 in 2 patients (nos. 6 and 10) and both genotypes in one patient (no. 11) (Fig. 6:1).

In the 8 HIV-infected patients from whom Z-genotyping was possible in both PBCs and tongue specimens, seven (nos. 1, 4, 8, 12, 17, 18, 19) were positive for the Z_2 genotype in the PBCs and tongue, while both Z_2 and Z_3 were detected in the PBCs and tongue of one patient (no. 11) (Fig. 6:1). The difference in the proportion of the two genotypes in the tongue and blood of the HIV-positive group was not significant (p=0.521). When the distribution of the two genotypes in the tongue were compared between the HIV-positive and HIV-negative groups, the difference was observed to be significant (p=0.024), while in the blood the difference was not significant (p=0.521). Table 6:1 summarises the distribution of the Z genotypes in the various groups.
For several patients, Z genotyping was negative for tongue or for blood, or for both specimens. This may be because some wild EBV variants do not possess the fragment that is amplifiable from the Bam HI Z region. We have noted our consistent inability to amplify this fragment from the Raji cell line, although the deletion in the Bam HI Z region in Raji cells, is not known to extend into the locus of amplification (Rickinson and Kieff, 1996).

**LMP and LMP<sub>del</sub> genotyping**

EBV variants may also be grouped according to whether or not they possess a 30-bp sequence in exon C of the LMP-1 encoding gene (Hu et al, 1991). Following PCR under the conditions described, a variant that carries the deletion in the LMP gene, forming the genotype designated as LMP<sub>del</sub>, is identified by gel electrophoresis as a 232-bp band (Fig. 6:2). The genotype without this deletion, designated LMP, yields a 262-bp PCR product (Fig. 6:2). LMP<sub>del</sub> was the predominant genotype in the individuals tested, whether HIV-infected or not, as 13 of 17 subjects from whom the LMP-1 gene product could be amplified from PBCs were exclusively LMP<sub>del</sub>, and 11 of 13 tongue specimens were LMP<sub>del</sub>-positive (Fig. 6:2). Three subjects (nos. 8, 20 and 46) were LMP-positive in the PBCs, two (nos. 8 and 20) were LMP-positive in the tongue, and one (no. 17) was positive for both LMP and LMP<sub>del</sub> in the PBCs. The differences in the distribution of the two genotypes, when compared between tongue and blood of either the HIV-positive or-negative group, or in either tongue or blood between each group, were not significant. The findings are summarised in Table 6:2.
A and B genotyping

Amplification with EBNA-3C primers under the conditions described, results in the production of a 153-bp fragment from type A viruses (eg. the B95-8 virus) and a 246-bp fragment from type B viruses (eg. the AG876 virus) (Fig. 6:3). PCR products could be obtained from 14 tongue specimens of the HIV-positive group but not from the controls, or from PBCs in either group, reflecting the poorer sensitivity of the non-nested PCR used for A/B typing. Seven specimens were type A, four type B and three types A plus B (Fig. 6:3).

Lack of correlation between genotypes in tongue specimens of HIV-infected patients.

The findings presented above show that, the distribution of Z genotypes in HIV-positive patients was independent of that of LMP-1 genotypes: there was no corresponding shift from blood to tongue in the relative frequency of LMP\textsubscript{del} to LMP; furthermore, the distribution of the Z genotypes was independent of that of LMP-1 genotypes. Further evidence of a lack of correlation between genotypes came from examining five HIV-infected patients, from whom amplicons from all three primer sets were obtained. Three of these (nos. 8, 18 and 19) were infected by EBV belonging to the Z\textsubscript{2}, LMP\textsubscript{del} and A genotypes, one (patient 17) by the Z\textsubscript{2}, LMP\textsubscript{del} and B genotypes, and one (patient 11) by Z\textsubscript{2} plus Z\textsubscript{3}, LMP\textsubscript{del} and A plus B genotypes.
Identity of $Z_2$ variants in the tongue to those in the blood of HIV-infected patients.

In view of the predominance of the $Z_2$ genotype in the tongue of most of the HIV-positive patients studied here, we attempted to determine if EBV variants bearing this genotype were common to those found in the circulation. The PCR primers that permit genotyping from the BZLF-1 gene also encompass hypervariable loci in the gene and we have demonstrated that an SSCP assay utilizing PCR products generated from these primers discriminates between naturally occurring EBV variants (Chapter 5). We applied this procedure to clones generated from the BZLF-1 gene derived from the tongue and PBCs of patients 1 and 8. In patient 1, the same banding pattern was observed in all 10 clones derived from PBCs and from the tongue (Fig. 6:4), suggesting that the variants in both compartments were identical. In patient 8, the banding pattern of two out of the four clones amplified from the tongue specimens was identical to 10 of 14 clones from the blood (Fig. 6:4).

Discussion

Following primary infection, EBV persists life-long, usually without clinical consequences. The epithelium, principally the oropharynx, has been regarded to be the principal site of persistence, serving as a reservoir from which B lymphocytes are infected and subsequently destroyed by cytotoxic T cells (Chen et al, 1996). However, EBV typing analyses of patients before and after bone
marrow transplant (Gratama et al, 1988) and histologic analyses of lymphoid tissue of patients with infectious mononucleosis (Anagnostopoulos et al, 1995), and of healthy people (Tao et al, 1995), support the notion that the site of persistence is in the haematopoietic system, specifically in B cells.

EBV may also exhibit intra-host tissue tropism. Early studies indicated that, laboratory EBV isolates were unable to infect epithelial cells, but this could have been because the isolates were previously adapted to B cells (Sixbey et al, 1983). More definitive evidence of tissue tropism comes from the study by Chen and co-workers, (1996), which showed that, the $Z_2$ genotype was detected in 88% of PBCs, 10% of the nasopharyngeal biopsies and 14% of the throat washings, while, by contrast, the $Z_3$ genotype was detected in 13% of PBLs, 88% of nasopharyngeal biopsies and 86% of throat washings. The results of this study showed a similar distribution of Z genotypes in the healthy control group (the $Z_2$ frequency rate was 100% in PBCs and that of $Z_3$ in tongue scrapings was 75%).

More importantly, however, a shift in the distribution of these genotypes was observed in the HIV-infected group: the $Z_2$ genotype, but not $Z_3$, was predominantly observed in the tongue specimens (at 88% and 12% frequencies, respectively), even though the frequency rate of the $Z_2$ genotype in PBCs (79%) remained similar to that of the subjects studied by Chen and co-workers (1996).

A limitation to this study of Z genotype distributions is the differential rate of BZLF-1 PCR positivity between blood and tongue specimens, such that data
were obtained from paired and unpaired specimens. As mentioned earlier, all extracts contained amplifiable DNA, and the set of primers that we used to amplify DNA from the BZLF-1 region was the most sensitive one, because various combinations of other primer sets, including sets that appear suitable for nested PCR, did not result in reproducible, specific PCR product bands in the agarose gel.

This study has found that in the HIV-infected group, the distribution of the Z genotypes was independent of that of the LMP genotypes: there was no corresponding shift from blood to tongue in the relative frequency of LMP_{del} to LMP genotype (LMP_{del} was predominant in PBCs of the controls). Chen and co-workers (1996), reported that the LMP_{del} genotype was equally distributed in both epithelial and peripheral blood compartments. Furthermore, in the present investigation, it was found that, in the tongue specimens of the HIV-positive group, the Z genotype distribution was also independent of that of A/B type, as patients infected in the tongue by type A virus could be infected by variants bearing either Z_{2} or Z_{3} genotype.

As EBV is very infrequently detected in the tongue of healthy hosts, the positive detection of the Z and LMP genotypes in tongue scrapings of the controls could have arisen from contamination by EBV shed elsewhere, eg. the pharynx. However, the frequent detection of these genotypes (in particular types A and B by the relatively insensitive non-nested PCR procedure) in tongue specimens of the HIV-infected group is more likely to reflect true lingual EBV infection.
It has been previously demonstrated by *in situ* hybridisation (Boulter *et al.*, 1996), that EBV undergoes permissive infection in the tongue early in HIV infection, before overt OHL develops. If the frequent detection of the Z2 genotype in the tongue is the result of true EBV infection, then the question arises as to why this shift should occur in HIV-infected hosts.

One possibility is the loss of one copy of the 29-bp repeat in the BZLF-1 intron after recombination with Z2 variants (Walling *et al.*, 1994). The other possibility is take-over following superinfection of the lingual epithelium by Z2 variants, with the elimination of the pre-existing Z3 variant. In either case, lingual Z2 variants could either originate from another host, or be carried by the blood from other compartments of the same host. Our study of clones derived from the BZLF-1 amplicons, showing that in HIV-infected patients, a proportion or all EBV variants in the tongue were identical to those in the blood, indicates the haematogenous carriage of Z2 genotype to the tongue, and points, further, to the endogenous provenance of Z2 genotype. It is conjectured that, in the face of immunosuppression, the activity of Z2-EBV activation is heightened, and this leads to an increased level of viral shedding from the endogenous site of persistence into the blood.

Baskin and co-workers (1995), reported that, rhesus monkeys infected by simian immunodeficiency virus could develop proliferative lesions histologically resembling OHL (the inclusion-bearing cells of which harbour EBV-like viruses) on the squamous epithelium at multiple body sites, not only the tongue. Such an
observation favours a model of OHL pathogenesis in which, hematogenous
dissemination of EBV plays a crucial role. The present findings provide support
for this model.
CHAPTER 7

ATTITUDES AND KNOWLEDGE OF UK DENTISTS AND DENTAL STUDENTS REGARDING HIV INFECTION AND AIDS PATIENTS
Introduction

Dental health care workers including dentists, hygienists, assistants and receptionists, have all been demonstrated to have some reluctance to treating HIV-infected patients (Hardie, 1992a; 1992b; Haring and Lind, 1992). It is accepted that, the dentist has a principal role in influencing members of his team (Hardie, 1992a), and that his/her attitude is, in turn, affected by the level of knowledge of HIV disease (Gerbert et al, 1988).


The aim of the present investigation was to provide more data regarding the knowledge of a substantial number of UK dentists and dental students pertaining to HIV disease. The dentists targeted for the study were in the younger age group, as it was felt that, this group would have had some formal education regarding HIV infection during their university years.
Materials and Methods

Seventy dentists with one or two years postgraduate experience (37 male; 33 female; median age 24 years, range 23 to 29 years) and 101 clinical dental students (46 male; 55 female; median age 21 years, range 19-31 years), attending a University Dental School and Hospital in UK, were requested to complete a self-administered questionnaire, that enquired into both their attitudes regarding the dental treatment of HIV-infected persons and their knowledge of HIV-infection (Appendix).

Results

All dental staff and students completed the questionnaire. The respondents attitudes to the dental treatment of HIV-infected individuals are outlined in Table 7:1; their answers to questions concerning knowledge of general aspects of HIV disease are summarised in Tables 7:2 and 7:3.

Discussion

Studies of medical health care staff indicate that, not all are willing to provide care for HIV-infected persons (Wertz et al, 1987; Gordin et al, 1987; Milne and Keen, 1988; Anderson and Mayon-White, 1988; Andre, 1988; Storosum et al, 1991; Roxburgh et al, 1992), although recent studies have demonstrated a more positive attitude towards this particular patient population (McDaniel et al, 1995; Carter et al, 1996; Fournier et al, 1997).

Dental students may be more anxious than medical students about treating HIV-infected patients (Bernstein et al, 1990; Weyant et al, 1994), and their willingness to treat such individuals may be influenced by the risk group of the patient, and
the clinical experience of the student (e.g., year of clinical training, frequency of
treating HIV-infected patients and location of training) (Yablon et al., 1989;
Wisborg and Brattebo, 1989; Cohen and Grace, 1989; Bernstein et al., 1990;
Solomon et al., 1991).

One study identified two major factors, which may account for the observed
differences between medical and dental students in desire to treat HIV-infected
patients (Weyant et al., 1994). One, is that dental students are at considerably
higher risk for exposure to blood and body fluids; consequently, the perception
of occupational risk of HIV infection is likely to be lower for medical
students. This hypothesis is supported by the fact, that medical students who
planned to enter high-risk specialties (i.e., surgical) reported less desire to treat
HIV-infected patients. A second factor is that, dental students select dental
school because, they are considerably less comfortable treating seriously ill
people (Weyant et al., 1994).

In the present investigation, up to 86% of young dentists and 85% of dental
students, were willing to treat symptomless HIV-infected patients, however, like
previous similar studies, this willingness fell to about 62% when respondents
were asked to treat patients with AIDS.

The willingness of the present group of students and staff is certainly better than
that of most previously examined groups of dental health care staff (Table 7:4),
and may reflect their early years of clinical practice, recent teachings in cross-
infection control or even their altruism.

Despite their willingness to treat HIV-infected persons, the majority of
respondents believed that, this might lead to a loss of non-HIV-infected patients,
loss of ancillary staff and possibly be financially unrewarding. Currently, there
is no evidence that, these are likely eventualities, however, it seems that dental
patients are unlikely to be drawn away from practices that treat HIV-infected
individuals (Gordin et al, 1987; Andre, 1988; Wisborg and Brattebo, 1989;
Scheutz, 1989; Gerbert et al, 1989a; 1989b; Bowden et al, 1989; Feldmann et al,
1990; Tesch et al, 1990; Ficarrotto et al, 1990; Solomon et al, 1991; Storosum et

The present staff and students generally agreed that, HIV-infected patients should
be treated in general dental practice, although would require referral to
appropriate centres for the diagnosis and management of HIV-related oral
problems, a view shared by medical specialists.

A further, reassuring aspect of the attitudes of respondents is their belief that,
direct questioning by dentists possibly supplemented by a questionnaire, would
be the most likely means of assessing a patient's HIV status, methods most likely
to obtain the optimal information concerning dental patient's medical history
(Porter et al, 1997). Perhaps reflecting their inexperience, 59% of dental
undergraduates indicated that, testing for HIV-antibodies might be advantageous,
whereas, only 11% of young dentists suggest HIV testing, as a practical method
of assessing dental patients.

Over 75% of responding dental staff and students believed, they had an adequate
knowledge to ensure that, they would not become infected with HIV.
Nevertheless, although over 82% were aware that condoms can prevent
transmission of HIV and 94% that, not all infected patients will have a positive
HIV test, less than 40% believed that, HIV infection ultimately leads to AIDS.
Almost all dentists, and 74% of students were aware that, the General Dental
Council of the UK does permit some HIV-infected staff to provide dental care
(General Dental Council, 1989), and almost all respondents realised that hepatitis B vaccination does not protect against HIV-infection.

Respondents were aware of the likely routes of transmission, but tended to underplay the importance of vaginal intercourse. In addition, there was some confusion concerning the likelihood of transmission of HIV via tattooing, possibly reflecting their knowledge that, HIV can be transmitted via sharps injuries, yet the lack of evidence that ear piercing and tattooing account for any instances of HIV transmission.

Similar studies among medical and dental health care workers have shown an inadequate knowledge regarding HIV/AIDS (Faris and Shouman, 1994). For example, over 90% of Indian dentists believed that the likelihood of HIV transmission in dentistry is very likely (Nair et al., 1995), 10% of medical students in Germany, estimated that, non-clinical situations like mosquito and animal bites or clinical situations like changing dirty linen or physical examination, as situations with a high risk of getting HIV infected (Klewewer and Kugler, 1996), and only 78% and 76% of hospital-based physicians in Canada and United States, respectively, understood the concept of a false-positive HIV-serologic test (Brachman et al., 1996). Indeed, most of the students from 15 out of 16 dental schools in UK, rated their teaching in the management of blood-borne virus carriers and the performance of barrier dentistry as inadequate (Gilbert and Nuttall, 1994).

However, recent studies indicated that, knowledge levels among medical and dental students were unrelated to their desire to treat, and that homophobia, aversion to intravenous drug users and concerns about social stigma were associated with decreased willingness, which, in turn suggest that educational interventions aimed simply at increasing the provider’s knowledge of HIV may
not be effective in changing behavior (Weyant et al, 1994; Rose, 1994; McDaniel et al, 1995; Carter et al, 1996; Zuber and Werner, 1996).

Despite the very positive attitudes of the present group of young UK dentists and dental students, there was a reluctance to treat patients with AIDS, which confirms the results of previous studies among UK dentists (Nattrass, 1988; Samaranayake et al, 1990; Porter et al, 1995; Hudson-Davies et al, 1995; Craven et al, 1996).

The knowledge that, not all UK dentists vaccinated against hepatitis B virus are willing to treat patients with hepatitis (Scully et al, 1990), suggests that, the development of an HIV vaccine may not improve the availability of dental care for HIV infected persons significantly.

Dental staff and students are at low risk of becoming infected with HIV, or nosocomially transmitting HIV to their patients or staff, as it is known that, HIV transmission between patient's and staff is unlikely (Porter and Scully, 1992; Capilouto et al, 1992; Tokars et al, 1993; Gerberding, 1996), and this group is unlikely to acquire HIV via sexual routes (Porter et al, 1991). Thus, we urgently need more dentists with specialised knowledge as regards the oral health of HIV-infected people (Robinson and Croucher, 1994; Robinson et al, 1994).
CHAPTER 8

PREVALENCE OF HIV-INFECTED PATIENTS ATTENDING A
DENTAL OUT-PATIENT CLINIC IN THE UNITED KINGDOM
Introduction

The prevalence of infection with Human Immunodeficiency Virus is increasing, particularly in the developing world (Scully, 1997). A total of 15,074 cases of AIDS have been reported in the United Kingdom between 1982 (when reporting began) and the end of 1997, 73% of whom are known or presumed to have died (Communicable Disease Report, 1998). Subsequently, many more HIV-infected persons will seek routine dental care. However, the prevalence of HIV infection in patients attending a UK dental unit appears not to have been detailed. Therefore, a group of patients attending a UK dental outpatient clinic were examined for evidence of serum antibodies to HIV.

Materials and Methods

Patient group

The study group comprised 255 patients, attending the Oral Medicine Department of the Eastman Dental Institute for Oral Health Care Sciences, University of London, during June 1997. Patients attended as a result of self-referrals or were referred by medical or dental general practitioners. Ninety eight of the group were male (38.4%) and 157 female (61.6%). The median age of the study population was 48 years (age-range 12-88 years). Serum samples were collected and tested for antibodies to HIV, in duplicate, using a dip-stick type ELISA Kit (Saliva Diagnostic Tests), and confirmed by Western Blot.
Results

Only one (0.4%) of the patient group was positive to ELISA testing, and this later gave rise to an equivocal result using Western Blot analysis. This patient was a, otherwise healthy, 12 year old Caucasian girl, whose parents refused all subsequent clinical follow-up.

Discussion

In the present study, serological evidence of HIV infection was detected in 1 of 255 patients (0.4%), attending an outpatient clinic in a large Dental Hospital in Central London.

There appear to have been very limited studies examining the sero-prevalence of HIV in dental patients, despite the fact that infection with HIV is increasing in most parts of the world. In San Francisco, the prevalence was estimated to be 4.8% in patients undergoing oral and maxillofacial surgery based on a questionnaire (Dodson et al, 1993). In a retrospective cohort study, in the USA, in which, the patient’s medical and dental records were reviewed to identify serostatus, 44 of the 145 dental patients were identified as HIV positive (30%) (Dodson et al, 1994), while a 2.8% positivity for HIV was demonstrated in a dental patient population, who were also positive for hepatitis B surface antigen (Cade et al, 1994). In a much earlier study, out of a group of 7090 dental patients at the University of Minnesota Dental School, 206 patients were considered to be at risk of HIV infection, using as criteria, the patients’ medical history, and intra
and extra-oral signs and symptoms, suggestive of HIV infection. Subsequently, however, only 11 (5.3%) of 206 and of 7090 patients tested HIV positive (Murrah and Scholtes, 1988).

In addition, small groups of patients treated for facial injuries have been tested in Canada (Hughes and Bailey, 1993) and Spain (Martinez-Gimeno et al, 1992), with reported HIV sero-prevalence of the rate of 2.2% and 14.7% respectively. Another study from UK found none of 100 patients with facial injuries to be HIV-infected (Crosher et al, 1997).

Recently, one study, using a salivary diagnostic test, found 1.2 % of a group of Brazilian patients to be HIV-infected (de Almeida et al, 1997). Salivary antibody testing, compared with serum testing, has the advantages of absence of needlestick injuries, simplicity, ease of collection, lack of need for trained staff, greater compliance in higher risk groups (Major et al, 1991), better acceptability by children, and less occupational hazard because of the low titers of HIV in the fluid (Barr et al, 1992; Young et al, 1993; Moore et al, 1993) and HIV inhibitory action of saliva (Yeh et al, 1992; Moore et al, 1993; Coates et al, 1991; Bergey et al, 1994). These factors, thus, reduce the occupational risks during sample collection.

Potentially, testing for HIV in a dental setting may be of some benefit, as asymptomatic HIV-infected persons can be identified and thus receive early effective treatment, adequate counselling and education in safe sexual practices.
However, the results of the present study would suggest that such testing is not likely to be of any advantage in communities with low prevalence rates of HIV disease, where detection of HIV-infection is unlikely, unless perhaps patients belong to well-defined risk groups such as homosexual males or injecting drug users. As few patients attending a UK dental clinic are likely to be unknowingly HIV-infected, the results of the present study also suggest that, UK dental health care staff are not at notable risk of nosocomial acquisition of HIV.
This thesis has investigated various oral and dental aspects of HIV disease:

Molecular epidemiologic studies were carried out in order to determine possible diversity of Epstein-Barr (EBV) strains or genotypes in HIV-infected hosts, and the biological implications of such diversity.

The extent of polymorphism in EBV DNA fragments amplified from the BamHI E, K, N and Z regions in EBV-carrying lymphoid cell lines was examined. Sequence dissimilarities in the fragment of the EBNA-3C gene specifying EBV genotypes 1 and 2 varied from 1 to 4% within each genotype; those in the first intron of the BZLF-1 gene were <2% within each genotype. Dissimilarities in the C-terminal unique domain of the EBNA-1 gene, and the fragment that encompasses and is upstream of the LMP-1 start codon, were genotype independent, varying between 2 to 7%. A non-isotopic single-strand conformation polymorphism procedure was developed and applied to fragments amplified from the BamHI K and N regions to directly monitor the diversity of EBV carried in tissues and body fluids. Extensive inter-host diversity was observed, whether or not, the host was co-infected with human immunodeficiency virus (HIV). In HIV-infected hosts, EBV diversity could be demonstrated between body sites, even in compartments that were anatomically proximate.

These results demonstrate that, variation in certain genomic segments of naturally occurring EBV can be extensive, extending beyond conventional
groupings based on dichotomous genotypic differences and the number of internal nucleotide repeats. Identification of hypermutable loci within the EBV genome, such as, those located in the BamHI K and N regions should permit individual EBV variants to be finely discriminated.

Oral and pharyngeal epithelium has been reported to preferentially carry EBV belonging to a genotype that possesses three copies of a 29-bp repeat in the first intron of the BZLF-1 gene, while peripheral blood mostly carries one that bears two copies. The present study, found that, while all study patients infected with HIV-1 without oral hairy leukoplakia (OHL), carried the genotype with two copies in peripheral blood, the majority of them also carried the same genotype in their tongue. The findings could not be fully correlated with EBV genotyped according to the A/B typing system or a grouping system based on a 30-bp deletion in the latent membrane protein-1 gene (LMP-1). Sequences of a proportion or all of the clones derived from the BZLF-1 amplicons in the tongue of HIV-1 infected patients were identical to those in blood. These findings are consistent with EBV hematogenous superinfection of the tongue of HIV-positive people. Further research is required to examine a larger number of specimens with overt oral hairy leukoplakia, to substantiate our findings.

The attitudes and knowledge of a group of UK dental staff and students regarding HIV disease were investigated. Up to 86% of young dentists and 85% of dental students were willing to treat symptomless HIV-infected patients. However, only about 60% were happy to treat patients with AIDS. The majority of respondents
beleived that, there was the possibility of loss of earnings, staff and patients, if it was known that a dental practice treated HIV-infected patients. Most agreed that, infected patients should receive routine dental care in general dental practice, although should be referred to an appropriate centre for evaluation of HIV-related problems. Seventy five percent of respondents beleived, they had an adequate knowledge of HIV infection, but when tested, this knowledge was incomplete.

Finally, using a serological diagnostic test, the prevalence of HIV among patients attending an inner-city Dental Hospital in London, was assessed and found to be 0.4%. The results suggest that very few patients attending a UK dental clinic are likely to be unknowingly HIV-infected and that UK dental health care staff are at negligible risk of nosocomial acquisition of HIV.


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Greenberg MS, Glick M, Nghiem L, Stewart JC, Hodinka R, Dubin G. (1997). Relationship of cytomegalovirus to salivary gland dysfunction in HIV-


Koletar SL, Russell JA, Fass RJ, Plouffe JF . (1990). Comparison of oral fluconazole and clotrimazole troches as treatment for oral candidiasis in patients...


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Kaposi sarcoma associated herpesvirus in peripheral blood of HIV-infected individuals and progression to Kaposi's sarcoma. Lancet 346: 799-802.


CHAPTER 11: TABLES
Table 1:1. Clinical features of HIV infection (based upon CDC 1993 revised classification system for HIV infection).

<table>
<thead>
<tr>
<th>Category A</th>
</tr>
</thead>
<tbody>
<tr>
<td>Asymptomatic HIV infection</td>
</tr>
<tr>
<td>Persistent generalised lymphadenopathy (PGL)</td>
</tr>
<tr>
<td>Acute (primary) HIV infection with accompanying illness or history of acute HIV infection</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Category B</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bacillary angiomatosis</td>
</tr>
<tr>
<td>Candidosis</td>
</tr>
<tr>
<td>- oropharyngeal</td>
</tr>
<tr>
<td>- vulvovaginal (persistent, frequent or poorly responsive to therapy)</td>
</tr>
<tr>
<td>- cervical dysplasia (moderate or severe)/cervical carcinoma <em>in situ</em></td>
</tr>
<tr>
<td>Fever (38.5°C) or diarrhoea lasting more than 1 month</td>
</tr>
<tr>
<td>Oral hairy leukoplakia</td>
</tr>
<tr>
<td>Herpes zoster (shingles) involving at least two distinct episodes or more than one dermatome</td>
</tr>
<tr>
<td>Idiopathic thrombocytopenic purpura</td>
</tr>
<tr>
<td>Listeriosis</td>
</tr>
<tr>
<td>Pelvic inflammatory disease, particularly if complicated by tubo-ovarian abscess</td>
</tr>
<tr>
<td>Peripheral neuropathy</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Category C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Candidosis of bronchi, trachea or lungs</td>
</tr>
<tr>
<td>Candidosis esophageal</td>
</tr>
<tr>
<td>Cervical cancer, invasive</td>
</tr>
<tr>
<td>Coccidioidomycosis, disseminated or extra-pulmonary</td>
</tr>
<tr>
<td>Cryptococcosis extra-pulmonary</td>
</tr>
<tr>
<td>Cryptosporidiosis, chronic intestinal (&gt;1 month’s duration)</td>
</tr>
<tr>
<td>Cytomegalovirus disease (other than liver, spleen or nodes)</td>
</tr>
<tr>
<td>Cytomegalovirus retinitis (with loss of vision)</td>
</tr>
<tr>
<td>Encephalopathy, HIV-related</td>
</tr>
<tr>
<td>Herpes simplex, chronic ulcer(s) (&gt;1 month’s duration): or bronchitis, pneumonitis or esophagitis</td>
</tr>
<tr>
<td>Histoplasmosis, disseminated or extra-pulmonary</td>
</tr>
<tr>
<td>Isosporiasis, chronic intestinal (&gt;1 month’s duration)</td>
</tr>
<tr>
<td>Kaposi’s sarcoma</td>
</tr>
<tr>
<td>Lymphoma, Burkitt’s (or equivalent term)</td>
</tr>
<tr>
<td>Lymphoma, immunoblastic (or equivalent term)</td>
</tr>
<tr>
<td>Lymphoma, primary of brain</td>
</tr>
<tr>
<td><em>Mycobacterium avium</em> complex or <em>M. kansasii</em>, disseminated or extra-pulmonary</td>
</tr>
<tr>
<td><em>Mycobacterium tuberculosis</em>, any site (pulmonary or extra-pulmonary)</td>
</tr>
<tr>
<td><em>Mycobacterium</em>, other species or unidentified species, disseminated or extra-pulmonary</td>
</tr>
<tr>
<td><em>Pneumocystis carinii</em> pneumonia</td>
</tr>
<tr>
<td>Pneumonia, recurrent</td>
</tr>
<tr>
<td>Progressive multifocal leukoencephalopathy</td>
</tr>
<tr>
<td><em>Salmonella</em> septicemia, recurrent</td>
</tr>
<tr>
<td>Toxoplasmosis of brain</td>
</tr>
<tr>
<td>Wasting syndrome due to HIV</td>
</tr>
</tbody>
</table>
Table 1:2. 1993 revised classification system for HIV infection and expanded AIDS surveillance case definition for adolescents and adults.

<table>
<thead>
<tr>
<th>CD4+T-cell categories</th>
<th>Clinical category A</th>
<th>Clinical category B</th>
<th>Clinical category C</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Asymptomatic, acute (primary) HIV or PGL</td>
<td>Symptomatic, not (A) or (C) conditions</td>
<td>AIDS-indicator conditions</td>
</tr>
<tr>
<td>(1) &gt; 500/l</td>
<td>A1</td>
<td>B1</td>
<td>C1</td>
</tr>
<tr>
<td>(2) 200-400/l</td>
<td>A2</td>
<td>B2</td>
<td>C2</td>
</tr>
<tr>
<td>(3) &lt;200/ l</td>
<td>A3</td>
<td>B3</td>
<td>C3</td>
</tr>
</tbody>
</table>
Table 1:3. Clinical manifestations of HIV disease.

<table>
<thead>
<tr>
<th>I. OPPORTUNISTIC INFECTIONS</th>
<th>II. NEOPLASMS</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Respiratory</strong></td>
<td><strong>Cutaneous</strong></td>
</tr>
<tr>
<td><em>Pneumocystis carinii</em></td>
<td><em>Candida albicans</em></td>
</tr>
<tr>
<td>Aspergillosis</td>
<td><em>Histoplasmosis</em></td>
</tr>
<tr>
<td>Candidosis</td>
<td><em>Papillomavirus</em></td>
</tr>
<tr>
<td>Zygomycosis</td>
<td><em>Herpes simplex</em></td>
</tr>
<tr>
<td>Strongyloidisis</td>
<td><em>Herpes zoster</em></td>
</tr>
<tr>
<td>Toxoplasmosis</td>
<td><em>Pox virus</em></td>
</tr>
<tr>
<td>Tuberculosis</td>
<td><em>Cryptococcus neoformans</em></td>
</tr>
<tr>
<td>Atypical mycobacterioses</td>
<td><em>Amoebiasis</em></td>
</tr>
<tr>
<td><em>Legionella</em></td>
<td><em>Staphylococcus</em></td>
</tr>
<tr>
<td><em>Klebsiella</em></td>
<td><em>Typical/atypical mycobacterioses</em></td>
</tr>
<tr>
<td><em>Pseudomonas aeruginosa</em></td>
<td><em>Ocular</em></td>
</tr>
<tr>
<td><em>Staphylococcus aureus</em></td>
<td><em>Cytomegalovirus</em></td>
</tr>
<tr>
<td><em>Streptococcus pneumoniae</em></td>
<td><em>Toxoplasmosis</em></td>
</tr>
<tr>
<td><em>Haemophilus influenzae</em></td>
<td><em>Disseminated</em></td>
</tr>
<tr>
<td><em>Cytomegalovirus</em></td>
<td><em>Atypical mycobacterioses</em></td>
</tr>
<tr>
<td>Oral and oesophageal</td>
<td><em>Cryptococcus neoformans</em></td>
</tr>
<tr>
<td><em>Candida albicans</em></td>
<td><em>Histoplasmosis</em></td>
</tr>
<tr>
<td>Histoplasmosis</td>
<td><em>Epstein-Barr virus</em></td>
</tr>
<tr>
<td><em>Cryptococcus neoformans</em></td>
<td><em>Cytomegalovirus</em></td>
</tr>
<tr>
<td>Atypical mycobacterioses</td>
<td><em>Adenovirus</em></td>
</tr>
<tr>
<td><em>Klebsiella pneumoniae</em></td>
<td><em>Coccidiodomycosis</em></td>
</tr>
<tr>
<td>Geotrichosis (rare)</td>
<td><em>Neural</em></td>
</tr>
<tr>
<td>Cat-scratch bacillus (rare)</td>
<td><em>Polyoma JC virus</em></td>
</tr>
<tr>
<td>Herpes simplex</td>
<td><em>Toxoplasma gondii</em></td>
</tr>
<tr>
<td>Herpes zoster</td>
<td><em>Papovavirus</em></td>
</tr>
<tr>
<td>Cytomegalovirus</td>
<td><em>Cryptococcus neoformans</em></td>
</tr>
<tr>
<td>Epstein-Barr virus</td>
<td></td>
</tr>
<tr>
<td>Papillomavirus</td>
<td></td>
</tr>
<tr>
<td>Actinomycosis</td>
<td></td>
</tr>
<tr>
<td><em>Salmonella enteritidis</em></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>III. NEUROLOGICAL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Subacute encephalitis</td>
</tr>
<tr>
<td>Peripheral neuropathies</td>
</tr>
<tr>
<td>Vascular myelopathy</td>
</tr>
<tr>
<td>Aseptic meningitis</td>
</tr>
<tr>
<td>Other</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>IV. OTHER</th>
</tr>
</thead>
<tbody>
<tr>
<td>Seborrhoeic dermatitis</td>
</tr>
<tr>
<td>Granuloma annulare-like eruptions</td>
</tr>
<tr>
<td>Thrombocytopenic purpura</td>
</tr>
<tr>
<td>Drug eruptions</td>
</tr>
</tbody>
</table>
Table 2.1. HIV-related oral lesions.

<table>
<thead>
<tr>
<th>MORE COMMON</th>
<th>LESS COMMON</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>I. INFECTIONS</strong></td>
<td><strong>I. INFECTIONS</strong></td>
</tr>
<tr>
<td><strong>Fungal</strong></td>
<td><strong>Fungal</strong></td>
</tr>
<tr>
<td>Acute pseudomembranous candidosis</td>
<td>Aspergillosis</td>
</tr>
<tr>
<td>Erythematous candidosis</td>
<td>Histoplasmosis</td>
</tr>
<tr>
<td>Hyperplastic candidosis</td>
<td>Zygomycosis</td>
</tr>
<tr>
<td>Angular cheilitis</td>
<td>Cryptococcus neoformans</td>
</tr>
<tr>
<td>Median rhomboid glossitis</td>
<td>Geotrichosis</td>
</tr>
<tr>
<td>Palatal papillary hyperplasia</td>
<td>Bacterial</td>
</tr>
<tr>
<td><strong>Bacterial</strong></td>
<td><strong>Mycobacterium avium-intracellulare</strong></td>
</tr>
<tr>
<td>Linear gingival erythema</td>
<td><em>Klebsiella pneumoniae</em></td>
</tr>
<tr>
<td>Necrotising ulcerative gingivitis</td>
<td><em>Enterobacter cloacae</em></td>
</tr>
<tr>
<td>Necrotising ulcerative periodontitis</td>
<td><em>Escherichia coli</em></td>
</tr>
<tr>
<td><strong>Viral</strong></td>
<td>Salmonella enteritidis</td>
</tr>
<tr>
<td>Oral hairy leukoplakia (Epstein-Barr Virus)</td>
<td>Epithelioid bacillary angiomatosis</td>
</tr>
<tr>
<td>Herpes simplex virus</td>
<td>Submandibular cellulitis</td>
</tr>
<tr>
<td>Varicella-Zoster</td>
<td>Treponema pallidum</td>
</tr>
<tr>
<td><strong>II. NEOPLASMS</strong></td>
<td>Actinomycosis</td>
</tr>
<tr>
<td>Kaposi’s sarcoma (HHV-8)</td>
<td>Necrotizing ulcerative stomatitis</td>
</tr>
<tr>
<td>Hodgkin’s lymphoma</td>
<td>Viral</td>
</tr>
<tr>
<td>Non-Hodgkin’s lymphoma</td>
<td>Human Papilloma Virus</td>
</tr>
<tr>
<td></td>
<td>Cytomegalovirus</td>
</tr>
<tr>
<td></td>
<td>Human herpes Virus 6, 7 and 8</td>
</tr>
<tr>
<td></td>
<td><strong>II. NEOPLASMS</strong></td>
</tr>
<tr>
<td></td>
<td>Hodgkin’s lymphoma</td>
</tr>
<tr>
<td></td>
<td>Squamous cell carcinoma</td>
</tr>
<tr>
<td></td>
<td><strong>III. NEUROLOGICAL</strong></td>
</tr>
<tr>
<td></td>
<td>Paraesthesia</td>
</tr>
<tr>
<td></td>
<td>Facial palsy</td>
</tr>
<tr>
<td></td>
<td>Hyperesthesia</td>
</tr>
<tr>
<td></td>
<td>Dysphagia</td>
</tr>
<tr>
<td></td>
<td><strong>IV. MISCELLANEOUS</strong></td>
</tr>
<tr>
<td></td>
<td>Recurrent aphthous ulceration</td>
</tr>
<tr>
<td></td>
<td>Progressive necrotising ulceration</td>
</tr>
<tr>
<td></td>
<td>Toxic epidermolysis</td>
</tr>
<tr>
<td></td>
<td>Delayed wound healing</td>
</tr>
<tr>
<td></td>
<td>Thrombocytopenia</td>
</tr>
<tr>
<td></td>
<td>Salivary gland disease</td>
</tr>
<tr>
<td></td>
<td>HIV-embryopathy</td>
</tr>
<tr>
<td></td>
<td>Hyperpigmentation</td>
</tr>
<tr>
<td></td>
<td>Granuloma annulare</td>
</tr>
<tr>
<td></td>
<td>Exfoliative cheilitis</td>
</tr>
<tr>
<td></td>
<td>Penicilliosis</td>
</tr>
</tbody>
</table>
Table 2:2. Management of HIV-related oral lesions (adapted from the British Dental Association, 1994).

<table>
<thead>
<tr>
<th>Lesion Type</th>
<th>Treatment Options</th>
</tr>
</thead>
</table>
| Pseudomembranous and erythematous candidosis:    | • Amphotericin lozenges 10,000 iu tabs. Dissolve 4-8 tabs slowly in mouth, daily for 2 weeks.  
• Nystatin 100,000 iu pastilles. Dissolve slowly in mouth, four times daily, for 2 weeks.  
• Miconazole oral gel. Hold 5 ml in mouth, three times daily, for 2 weeks.  
• Ketoconazole tabs 200 mg. 1 tab per day, for 2 weeks.  
• Fluconazole 50 mg tabs. 1 tab per day, for 2 weeks.  
• Itraconazole 100 mg. 1 tab per day, for 2 weeks. |
| Oral Hairy Leukoplakia:                          | see Table 3:6.                                         |
| Recurrent herpes simplex virus (HSV) infection:  | • Acyclovir 200 or 400 mg tabs. 1 tab, 5 times daily, for 5 days. |
| Cytomegalovirus (CMV) infection:                | • Intra-venous therapy with ganciclovir or foscarnet.  |
| Linear gingival erythema:                       | • Scaling of affected areas. Chlorhexidine mouth rinse. |
| Necrotising ulcerative gingivitis, periodontitis, or stomatitis | • Debride affected areas and irrigate with chlorhexidine solution.  
• Oral hygiene instruction, scaling and polishing and reviews to prevent relapse.  
• Metronidazole 200mg tabs. 1 tab, three times daily, for 3 days.  
• Chlorhexidine gluconate 0.2%, 250ml bottle. Rinse with 10ml, for 1 minute twice daily. |
Kaposi's sarcoma:

- Radiotherapy, chemotherapy and/or surgical excision.
- Lesions on the gingivae can be reduced by meticulous oral hygiene.
- Vinblastine sulphate 0.1 mg/ml per cm² of lesion.
- 1 % or 3% sodium tetradecyl sulphate. 0.1 ml per cm² of lesion.

Non-Hodgkin’s lymphoma:

- Radiotherapy or chemotherapy. The lesions occasionally regress spontaneously.

Ulceration “Not Otherwise Specified”:

- Hydrocortisone 2.5mg lozenges. Hold against ulcer 4 times daily.
- Triamcinolone dental paste. 0.1% in an adhesive base. Apply to ulcer 4 times daily.
- Prednisolone 5mg tabs. 1 tab, three times per day, for seven days, tailing of exponentially.
- Systemic glucocorticosteroid therapy should only be administered in collaboration with the patient’s physician.

Salivary gland disease:

- Stimulate saliva: sugar-free chewing gum.
- Topical fluoride application to prevent caries.
- Frequent sips of water (but not drinks with sugar).
- Avoid alcohol, tea, coffee and other caffeine-containing drinks.
- Artificial saliva [eg. Luborant®].

1. Typically presents as unilateral or bilateral, adherent, slightly elevated whitish or grey patches
2. Principally located mainly on lateral margins, dorsum or ventrum of the tongue
3. Occasionally observed over the floor of the mouth, palate or oropharynx
4. Vertically corrugated appearance
5. Usually asymptomatic
**TABLE 3:2. Histological features of Oral Hairy Leukoplakia.**

<p>| 1. Hyperparakeratosis          |
| 2. Acanthosis                  |
| 3. Keratinized “hair-like” projections |
| 4. Koilocytosis with pyknotic nuclei and perinuclear halos in the prickle cell layer |
| 5. Intranuclear inclusions     |
| 6. Few or no Langerhan’s cells |
| 7. Limited or absent inflammatory cell infiltrate in the lamina propria |</p>
<table>
<thead>
<tr>
<th>Report</th>
<th>Cases</th>
<th>General Diagnosis</th>
<th>Diagnosis of HL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Itin et al, 1988</td>
<td>1</td>
<td>RT</td>
<td>Histology, Southern blot, DNA in situ hybridization, electron microscopy</td>
</tr>
<tr>
<td>Epstein et al, 1988</td>
<td>2</td>
<td>BMT</td>
<td>Histology</td>
</tr>
<tr>
<td>Greenspan et al, 1989</td>
<td>1</td>
<td>RT</td>
<td>Histology, in situ hybridization</td>
</tr>
<tr>
<td>Birek et al, 1989</td>
<td></td>
<td></td>
<td>Histology</td>
</tr>
<tr>
<td>Syijanen et al, 1989</td>
<td>1</td>
<td>AML</td>
<td>Histology, in situ hybridization</td>
</tr>
<tr>
<td>MacLeod et al, 1990</td>
<td>1</td>
<td>RT</td>
<td>Histology, in situ hybridization, electron microscopy</td>
</tr>
<tr>
<td>Reggiani and Pauluzzi, 1990</td>
<td>1</td>
<td>LT</td>
<td>Clinical features, serology</td>
</tr>
<tr>
<td>Schmidt-Westhausen et al, 1990</td>
<td>1</td>
<td>CT</td>
<td>Histology, electron microscopy, serology, immunostaining</td>
</tr>
<tr>
<td>Ficarra et al, 1991</td>
<td>1</td>
<td>MS</td>
<td>Histology, electron microscopy, in situ hybridization</td>
</tr>
<tr>
<td>Kanitakis et al, 1991</td>
<td>1</td>
<td>RT</td>
<td>Histology, electron microscopy, immunostaining</td>
</tr>
<tr>
<td>Epstein et al, 1993</td>
<td>10</td>
<td>BMT</td>
<td>Histology, electron microscopy (2/10), in situ hybridization (3/10)</td>
</tr>
<tr>
<td>Schmidt-Westhausen et al, 1993</td>
<td>3</td>
<td>LT</td>
<td>electron microscopy</td>
</tr>
<tr>
<td>Fluckiger et al, 1994</td>
<td>1</td>
<td>CT</td>
<td>Histology, electron microscopy</td>
</tr>
<tr>
<td>King et al, 1994</td>
<td>18</td>
<td>RT</td>
<td>Clinical features, histology</td>
</tr>
<tr>
<td>Schiodt et al, 1995</td>
<td>1</td>
<td>CT</td>
<td>Histology, in situ hybridization, serology</td>
</tr>
<tr>
<td>Lozada-Nur et al, 1995</td>
<td>2</td>
<td>CT</td>
<td>Histology, in situ hybridization</td>
</tr>
<tr>
<td>Kaminsky et al, 1995</td>
<td>1</td>
<td>RF/A-F-D</td>
<td>Histology, electron microscopy</td>
</tr>
</tbody>
</table>

RT: Renal Transplant, BMT: Bone Marrow Transplant, AML: Acute Myeloblastic Leukaemia, LT: Liver Transplant, CT: Corticosteroid Therapy, MS: Myeloblastic Syndrome, RF/A-F-D: Renal Failure/Anderson-Fabry’s Disease

| 1. Idiopathic leukoplakia          |
| 2. Smoker’s keratosis             |
| 3. Frictional keratosis           |
| 4. Acute pseudomembranous candidosis |
| 5. Chronic hyperplastic candidosis |
| 6. “Plaque-like” type of lichen planus |
| 7. Lichenoid reaction             |
| 8. Erythema migrans               |
| 9. White sponge naevus            |
| 10. Oral graft-versus-host disease |
TABLE 3.5. Diagnosis of Oral Hairy Leukoplakia.

A. Provisional Diagnosis
- Characteristic gross appearance (see Table 3:1) with or without
- Non-responsiveness to antifungal therapy

B. Presumptive Diagnosis
- Light microscopy of histologic sections: hyperkeratosis, koilocytosis, acanthosis and absence of inflammatory cell infiltrate
  or
- Light microscopy of cytological preparations: nuclear beading and chromatin margination

C. Definitive Diagnosis
- *In situ* hybridization of histologic or cytologic specimens: positive staining for EBV-DNA
  or
- Electron microscopy of histologic or cytologic specimens: herpes-like particles

<table>
<thead>
<tr>
<th>Report</th>
<th>Mode of therapy</th>
<th>Dose</th>
<th>Time to respond</th>
<th>Remission Time</th>
<th>Side effects</th>
</tr>
</thead>
<tbody>
<tr>
<td>Friedman-Kien, 1986</td>
<td>ACV</td>
<td>800mg QDS for 2/52</td>
<td>5-8 d</td>
<td>2-8/52</td>
<td>NR</td>
</tr>
<tr>
<td>Schofer et al, 1987</td>
<td>ACV</td>
<td>7.5mg/Kg TDS for 1/52 and 400mgX 5/d</td>
<td>7 d</td>
<td>3/52</td>
<td>NR</td>
</tr>
<tr>
<td>Resnic et al, 1988 Brockmeyer et al, 1989</td>
<td>ACV</td>
<td>3.2 g/d for 20 d</td>
<td>14-28d</td>
<td>14-48 d</td>
<td>nausea</td>
</tr>
<tr>
<td>Kessler et al, 1988</td>
<td>AZT</td>
<td>200mg X 6/d</td>
<td>7-49d</td>
<td>NR</td>
<td>NR</td>
</tr>
<tr>
<td>Phelan and Klein, 1988</td>
<td>AZT</td>
<td>200mgX6/d</td>
<td>42d</td>
<td>NR</td>
<td>NR</td>
</tr>
<tr>
<td>Greenspan et al, 1990</td>
<td>Descicl</td>
<td>250mg TDS for 2/52</td>
<td>7-28d</td>
<td>4-16/52</td>
<td>tachycardia, Head, neck and jaw aches</td>
</tr>
<tr>
<td>Albrecht et al, 1994</td>
<td>Foscarn</td>
<td>4800mg-6000mg BS</td>
<td>14-21d</td>
<td>NR</td>
<td>NR</td>
</tr>
<tr>
<td>Lozada-Nur et al, 1991</td>
<td>PRS 25%</td>
<td>Lesional application for 30-60s</td>
<td>4-5d</td>
<td>2-28/52</td>
<td>NR</td>
</tr>
<tr>
<td>Sanchez et al, 1992</td>
<td>PRS 25%</td>
<td>„</td>
<td>3-5d</td>
<td>16,36/52</td>
<td>sore tongue, altered taste as above, plus burning</td>
</tr>
<tr>
<td>Lozada-Nur et al, 1992</td>
<td>PRS 25%</td>
<td>„</td>
<td>7d</td>
<td>2-28/52</td>
<td>sore tongue, altered taste as above, plus burning</td>
</tr>
<tr>
<td>Gowdey et al, 1995</td>
<td>PRS 25%</td>
<td>„</td>
<td>2-30d</td>
<td>NR</td>
<td>NR</td>
</tr>
</tbody>
</table>

ACV: acyclovir, AZT: azithothymidine, PRS: podophyllum resin solution, QDS: four times daily, TDS: three times daily, d: day, s: second, NR: non reported
TABLE 5:1. Nucleotide sequences and coordinates of primers used in the study.

<table>
<thead>
<tr>
<th>Primers</th>
<th>Nucleotide sequence</th>
<th>Coordinate of 5’ end*</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>BamHI K region</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>outer, sense</td>
<td>5’-TGATGGAGGCAGGCAGCGCAAAAAAAG-3’</td>
<td>109,311</td>
</tr>
<tr>
<td>outer, anti-sense</td>
<td>5'-GAAACCAGGGAGGCAATCTACT-3'</td>
<td>109,780</td>
</tr>
<tr>
<td>inner, sense</td>
<td>5’-CGCAAAAAAAGGAGGTTGTTT-3’</td>
<td>109,324</td>
</tr>
<tr>
<td>inner, anti-sense</td>
<td>5’-CATCGTCAAAGCTGCACACAG-3’</td>
<td>109,756</td>
</tr>
<tr>
<td><strong>BamHI N region</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>sense</td>
<td>5’-GAGAAGGAGAGCAAGGCCTA-3’</td>
<td>169,382</td>
</tr>
<tr>
<td>anti-sense</td>
<td>5’-CGCAGGCTGCTTTTCCATTCC-3’</td>
<td>169,779</td>
</tr>
<tr>
<td>heminested, sense</td>
<td>5’-AAGGCCTAGGGAAGAGGAGA-3’</td>
<td>169,394</td>
</tr>
<tr>
<td><strong>BamHI Z region</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>sense</td>
<td>5’-CGCAGCCCTGATTTTTCAGA-3’</td>
<td>102,304</td>
</tr>
<tr>
<td>anti-sense</td>
<td>5’-CAACAGCCAGAATCGTAAG-3’</td>
<td>102,669</td>
</tr>
<tr>
<td><strong>BamHI E region</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>sense</td>
<td>5’-AGAAGGGGAGCGTTGTTGTG-3’</td>
<td>99,939</td>
</tr>
<tr>
<td>anti-sense</td>
<td>5’-GGCTCGTTTGTGACGTCGCC-3’</td>
<td>100,091</td>
</tr>
</tbody>
</table>

* Coordinates correspond to the sequence of the B95-8 EBV genome.
TABLE 6:1. Nucleotide sequences and coordinates of primers used in study.

<table>
<thead>
<tr>
<th>Primers</th>
<th>Nucleotide sequence</th>
<th>Coordinate of 5' end*</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>BZLF-1 gene</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>sense</td>
<td>5'-CGCAGCCTGTCATTTTCAGA-3'</td>
<td>102,304</td>
</tr>
<tr>
<td>anti-sense</td>
<td>5'-CAACAGCCAGAATCGGTAAG-3'</td>
<td>102,669</td>
</tr>
<tr>
<td>heminested, sense</td>
<td>5'-TGATTTGCGAGCAGCCACCT-3'</td>
<td>102,324</td>
</tr>
<tr>
<td><strong>LMP-1 gene</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>outer, sense</td>
<td>5'-GTAAATGGAGGGAGAGTCAGTC-3'</td>
<td>168,096</td>
</tr>
<tr>
<td>outer, anti-sense</td>
<td>5'-GTTCAGCGATCCCCGATACTC-3'</td>
<td>168,401</td>
</tr>
<tr>
<td>inner, sense</td>
<td>5'-TCAGGCAAGCCTATGACATGG-3'</td>
<td>168,116</td>
</tr>
<tr>
<td>inner, anti-sense</td>
<td>5'-GGAGGTAGTAAAGGTCGTCTC-3'</td>
<td>168,385</td>
</tr>
<tr>
<td><strong>EBNA-3C gene</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>sense</td>
<td>5'-AGAAGGGGAGCGTGTGTGTG-3'</td>
<td>99,939</td>
</tr>
<tr>
<td>anti-sense</td>
<td>5'-GGCTCGTTTTTGACGTGGCC-3'</td>
<td>100,091</td>
</tr>
</tbody>
</table>

* Coordinates correspond to the sequence of the B95-8 EBV genome.
TABLE 6:2. Distribution of $Z_2$ and $Z_3$ genotypes in tongue scrapings and Peripheral Blood Cells (PBCs) of HIV$^+$ and HIV$^-$ patients.

<table>
<thead>
<tr>
<th>Site</th>
<th>HIV$^+$</th>
<th></th>
<th>HIV$^-$</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$Z_2$</td>
<td>$Z_3$</td>
<td>$Z_2/Z_3$</td>
<td>Total</td>
</tr>
<tr>
<td>Tongue</td>
<td>7 (88%)</td>
<td>0</td>
<td>1 (12%)</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>1 (25%)</td>
<td>3 (75%)</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>Blood</td>
<td>11 (79%)</td>
<td>2 (14%)</td>
<td>1 (7%)</td>
<td>14</td>
</tr>
<tr>
<td></td>
<td>7 (100%)</td>
<td>0</td>
<td>7</td>
<td></td>
</tr>
</tbody>
</table>

P values for differences: tongue vs blood in HIV$^+$ group, p=0.521; tongue vs blood in HIV$^-$ group, p=0.024; HIV$^+$ vs HIV$^-$ groups for tongue, p=0.024; HIV$^+$ vs HIV$^-$ groups for blood, p=0.521. (Fisher’s exact test, 2-tailed; observations in the mixed infection ($Z_2+Z_3$) cells were excluded in the analysis.)
TABLE 6.3. Distribution of LMP and LMP$_{del}$ genotypes in tongue scrapings and Peripheral Blood Cells (PBCs) of HIV$^+$ and HIV$^-$ patients.

<table>
<thead>
<tr>
<th>Site</th>
<th>HIV$^+$</th>
<th>HIV$^-$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>LMP</td>
<td>LMP$_{del}$</td>
</tr>
<tr>
<td>Tongue</td>
<td>2 (17%)</td>
<td>10 (83%)</td>
</tr>
<tr>
<td>Blood</td>
<td>2 (17%)</td>
<td>9 (75%)</td>
</tr>
</tbody>
</table>

No significant differences in distribution of tongue and blood in HIV$^+$ group, of tongue and blood in HIV$^-$ group, of tongue between HIV$^+$ and HIV$^-$ groups, and of blood between HIV$^+$ and HIV$^-$ groups (p=1.000). Observations in the mixed infection (LMP+LMP$_{del}$) cells were excluded from the analysis.
Table 7:1. Attitudes of UK dentists and students regarding the dental treatment of HIV-infected persons.

<table>
<thead>
<tr>
<th></th>
<th>Under-graduates</th>
<th>Dentists</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Willing to treat HIV-infected individuals</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Symptomless HIV-disease</td>
<td>85%</td>
<td>86%</td>
<td>85.5%</td>
</tr>
<tr>
<td>ARC-like illness</td>
<td>80%</td>
<td>71.4%</td>
<td>75.7%</td>
</tr>
<tr>
<td>Patients with AIDS</td>
<td>62%</td>
<td>62.8%</td>
<td>62.4%</td>
</tr>
<tr>
<td>2. Undertaking the routine dental treatment of HIV-infected individuals in general dental practice</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Is likely to be financially unrewarding</td>
<td>35%</td>
<td>60%</td>
<td>47.5%</td>
</tr>
<tr>
<td>May result in loss of regular, non-infected patients</td>
<td>69%</td>
<td>70%</td>
<td>69.5%</td>
</tr>
<tr>
<td>May cause the dentist to gain the reputation of being homosexual</td>
<td>14%</td>
<td>15.7%</td>
<td>14.8%</td>
</tr>
<tr>
<td>May result in the loss of ancillary/nursing staff</td>
<td>72%</td>
<td>62.8%</td>
<td>67.4%</td>
</tr>
<tr>
<td>3. In general dental practice HIV-infected patients should</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Have all oral care undertaken by his/her dentist</td>
<td>63.5%</td>
<td>58.5%</td>
<td>56%</td>
</tr>
<tr>
<td>Be referred to a specialist dental unit (eg local dental school or oral surgery unit) for all dental treatment</td>
<td>35%</td>
<td>25.3%</td>
<td>30.1%</td>
</tr>
<tr>
<td>Only be referred to a specialist unit for the diagnosis and management of HIV-related oral problems</td>
<td>67%</td>
<td>77.6%</td>
<td>72.3%</td>
</tr>
<tr>
<td>4. How would/do you assess the status of patients?</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Using a questionnaire filled in by the dentist</td>
<td>61%</td>
<td>47.1%</td>
<td>54%</td>
</tr>
<tr>
<td>Direct questioning of the patient by the dentist</td>
<td>76%</td>
<td>68.6%</td>
<td>72.3%</td>
</tr>
</tbody>
</table>

277
<table>
<thead>
<tr>
<th></th>
<th>Undergraduates</th>
<th>Dentists</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Direct questioning of the patient by the nurse or receptionist</td>
<td>5%</td>
<td>0%</td>
<td>2.5%</td>
</tr>
<tr>
<td>Appearance and/or demeanor of patient</td>
<td>44%</td>
<td>27.1%</td>
<td>35.5%</td>
</tr>
<tr>
<td>HIV-testing</td>
<td>59%</td>
<td>11.4%</td>
<td>35.2%</td>
</tr>
<tr>
<td>Test for lymphopenia</td>
<td>2%</td>
<td>1.4%</td>
<td>1.7%</td>
</tr>
<tr>
<td>I do not*</td>
<td>0</td>
<td>23%</td>
<td>23%</td>
</tr>
<tr>
<td>Other</td>
<td>8%</td>
<td>18.6%</td>
<td>13.3%</td>
</tr>
</tbody>
</table>

*only dentists asked
Table 7.2. Knowledge of UK dentists and students regarding HIV-infection.

<table>
<thead>
<tr>
<th>Statement</th>
<th>Undergraduates</th>
<th>Dentists</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>i) I have an adequate knowledge of HIV infection that will ensure that I will not become infected with HIV.</td>
<td>76.2%</td>
<td>78.5%</td>
<td>77.3%</td>
</tr>
<tr>
<td>ii) All HIV infected individuals develop AIDS</td>
<td>36.6%</td>
<td>40%</td>
<td>38.3%</td>
</tr>
<tr>
<td>iii) Condoms prevent transmission of HIV during sexual intercourse</td>
<td>79.2%</td>
<td>85.7%</td>
<td>82.4%</td>
</tr>
<tr>
<td>iv) All HIV-infected individuals will have a positive HIV test result</td>
<td>10%</td>
<td>1.4%</td>
<td>5.7%</td>
</tr>
<tr>
<td>v) The General Dental Council (GDC) has stated that HIV-infected dentists can no longer practice dentistry</td>
<td>26%</td>
<td>4.2%</td>
<td>15.1%</td>
</tr>
<tr>
<td>vi) Testing for HIV infection requires consent</td>
<td>88%</td>
<td>90%</td>
<td>89%</td>
</tr>
<tr>
<td>vii) Oral manifestations of HIV infection are common</td>
<td>85.1%</td>
<td>78.6%</td>
<td>81.8%</td>
</tr>
<tr>
<td>viii) Hepatitis B vaccination may protect against HIV</td>
<td>3%</td>
<td>0%</td>
<td>1.5%</td>
</tr>
<tr>
<td>ix) All HIV-infected individuals should be quarantined</td>
<td>12.9%</td>
<td>0%</td>
<td>6.4%</td>
</tr>
</tbody>
</table>
Table 7:3. Knowledge of transmission of HIV infection.

<table>
<thead>
<tr>
<th>Transmission of HIV is highly likely via:</th>
<th>Under-graduates</th>
<th>Dentists</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Attending the hairdresser</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
</tr>
<tr>
<td>Social kissing</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
</tr>
<tr>
<td>Tears</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
</tr>
<tr>
<td>Sharing cups</td>
<td>2%</td>
<td>1.4%</td>
<td>1.7%</td>
</tr>
<tr>
<td>Vaginal intercourse</td>
<td>67.3%</td>
<td>72.8%</td>
<td>70%</td>
</tr>
<tr>
<td>Sharing razors</td>
<td>44.5%</td>
<td>20%</td>
<td>32.2%</td>
</tr>
<tr>
<td>Sharing toothbrushes</td>
<td>19.8%</td>
<td>1.4%</td>
<td>10.6%</td>
</tr>
<tr>
<td>Anal intercourse</td>
<td>96%</td>
<td>95%</td>
<td>95.5%</td>
</tr>
<tr>
<td>Tattooing</td>
<td>62.3%</td>
<td>35.7%</td>
<td>49%</td>
</tr>
<tr>
<td>Oral sex</td>
<td>36.6%</td>
<td>0%</td>
<td>18.3%</td>
</tr>
<tr>
<td>Attending a chiropodist</td>
<td>1.9%</td>
<td>11.4%</td>
<td>6.7%</td>
</tr>
<tr>
<td>Breast feeding</td>
<td>19.8%</td>
<td>41.4%</td>
<td>30.6%</td>
</tr>
<tr>
<td>Blood transfusions</td>
<td>56.4%</td>
<td>94.3%</td>
<td>75.3%</td>
</tr>
<tr>
<td>Sharing needles</td>
<td>98%</td>
<td>94.3%</td>
<td>96.1%</td>
</tr>
<tr>
<td>Insect bites</td>
<td>4.9%</td>
<td>1.4%</td>
<td>3.1%</td>
</tr>
<tr>
<td>Ear piercing</td>
<td>21.7%</td>
<td>4.3%</td>
<td>1.3%</td>
</tr>
<tr>
<td>Swimming pools</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
</tr>
<tr>
<td>Mutual masturbation</td>
<td>2.9%</td>
<td>0%</td>
<td>1.4%</td>
</tr>
<tr>
<td>Wet kissing (french kissing)</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
</tr>
</tbody>
</table>

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Table 7.4. Reported willingness of dentists and dental students to treat HIV-infected patients.

<table>
<thead>
<tr>
<th>Year</th>
<th>Region</th>
<th>% willing to treat</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>1986</td>
<td>Minnesota</td>
<td>23</td>
<td>DiAngelis et al., 1989</td>
</tr>
<tr>
<td>1986</td>
<td>USA</td>
<td>21</td>
<td>Verrusio et al., 1989</td>
</tr>
<tr>
<td>1987</td>
<td>USA</td>
<td>44</td>
<td>Aitchison et al., 1987</td>
</tr>
<tr>
<td>1987</td>
<td>Minnesota</td>
<td>37</td>
<td>DiAngelis et al., 1989</td>
</tr>
<tr>
<td>1987</td>
<td>New York</td>
<td>28</td>
<td>Yablon et al., 1989</td>
</tr>
<tr>
<td>1987</td>
<td>Chicago</td>
<td>68</td>
<td>Moretti et al., 1989</td>
</tr>
<tr>
<td>1988</td>
<td>San Francisco</td>
<td>23</td>
<td>Nattrass, 1988</td>
</tr>
<tr>
<td>1988</td>
<td>Chicago</td>
<td>23</td>
<td>Tofani et al., 1990</td>
</tr>
<tr>
<td>1988</td>
<td>USA</td>
<td>31</td>
<td>Verrusio et al., 1989</td>
</tr>
<tr>
<td>1988</td>
<td>UK</td>
<td>45</td>
<td>Nattrass, 1988</td>
</tr>
<tr>
<td>1988</td>
<td>Denmark</td>
<td>56</td>
<td>Scheutz, 1988</td>
</tr>
<tr>
<td>1989</td>
<td>Chicago</td>
<td>98</td>
<td>Hazelkorn, 1989</td>
</tr>
<tr>
<td>1989</td>
<td>San Francisco</td>
<td>89</td>
<td>Gerbert et al., 1989</td>
</tr>
<tr>
<td>1989</td>
<td>Los Angeles</td>
<td>82*</td>
<td>Samaranayake et al., 1990</td>
</tr>
<tr>
<td>1989</td>
<td>UK</td>
<td>74*</td>
<td>Samaranayake et al., 1990</td>
</tr>
<tr>
<td>1989</td>
<td>Texas</td>
<td>34</td>
<td>Dove et al., 1990</td>
</tr>
<tr>
<td>1989</td>
<td>Maryland</td>
<td>31-58</td>
<td>Grace and Cohen, 1993</td>
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<tr>
<td>1990</td>
<td>Pennslyvania</td>
<td>70</td>
<td>Rydman et al., 1990</td>
</tr>
<tr>
<td>1990/91</td>
<td>UK</td>
<td>48</td>
<td>Hudson-Davies et al., 1995</td>
</tr>
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<td>1991</td>
<td>USA</td>
<td>60</td>
<td>Sadowsky and Kunzel, 1991</td>
</tr>
<tr>
<td>1991</td>
<td>Israel</td>
<td>26</td>
<td>Trieger et al., 1993</td>
</tr>
<tr>
<td>Year</td>
<td>Location</td>
<td>Percentage</td>
<td>Source</td>
</tr>
<tr>
<td>------</td>
<td>----------</td>
<td>------------</td>
<td>--------</td>
</tr>
<tr>
<td>1992</td>
<td>Texas</td>
<td>42</td>
<td>Rankin et al., 1993</td>
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<tr>
<td>1992/93</td>
<td>Denmark</td>
<td>79</td>
<td>Scheutz and Langebaek, 1995</td>
</tr>
<tr>
<td>1993</td>
<td>Brazil</td>
<td>44</td>
<td>Sposto et al., 1994</td>
</tr>
<tr>
<td>1993</td>
<td>Italy</td>
<td>65</td>
<td>Angelillo et al., 1994</td>
</tr>
<tr>
<td>1993</td>
<td>USA</td>
<td>68</td>
<td>Bennett et al., 1995</td>
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<tr>
<td>1993</td>
<td>UK</td>
<td>60</td>
<td>Porter et al., 1995</td>
</tr>
<tr>
<td>?</td>
<td>Canada</td>
<td>67</td>
<td>McCarthy and MacDonald, 1996</td>
</tr>
<tr>
<td>?</td>
<td>UK</td>
<td>27</td>
<td>Craven et al., 1996</td>
</tr>
</tbody>
</table>

**DENTAL STUDENTS**

<table>
<thead>
<tr>
<th>Year</th>
<th>Location</th>
<th>Percentage</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>1989</td>
<td>Los Angeles</td>
<td>82*</td>
<td>Samaranayake et al., 1990</td>
</tr>
<tr>
<td>1989</td>
<td>Glasgow</td>
<td>74*</td>
<td>Samaranayake et al., 1990</td>
</tr>
<tr>
<td>1989</td>
<td>New York</td>
<td>6-79</td>
<td>Bernstein et al., 1990</td>
</tr>
<tr>
<td>1991</td>
<td>USA</td>
<td>62+</td>
<td>Solomon et al., 1991</td>
</tr>
<tr>
<td>1992</td>
<td>USA</td>
<td>45</td>
<td>Ranklin et al., 1993</td>
</tr>
</tbody>
</table>

* Combined dental faculty staff and students

+ Includes willingness to treat patients infected with hepatitis B virus (HBV)
CHAPTER 12 : FIGURES
Fig 5:1. Nucleotide sequence alignments of inter-primer DNA segments amplified from the BamHI E (A), Z (B), N (C) and K (D) regions of the EBV genome. Sequence of B95-8 virus is shown in full. Dots indicate identity, and dashes denote deletion, in relation to B95-8 bases positioned above. Figures to left indicate coordinates (relative to the B95-8 viral genome).
Fig 5.2. Comparison of banding patterns of PCR products amplified from 4 regions of the EBV genome carried in various lymphoid cell lines, following ethidium bromide-stained agarose gel electrophoresis (upper panels), and SYBR GREEN II-stained non-denaturing polyacrylamide gel electrophoresis conducted after heat denaturation of the PCR products (lower panels).

A. BamHI E; B. BamHI Z; C. BamHI N; and D. BamHI K. Lane markings: M = molecular weight markers (figures to left denote base-pair lengths); 1 = B95-8; 2 = EB 176; 3 = EB 185; 4 = P3HR-1; 5 = AW Ramos; 6 = AG876; 7 = Namalwa; 8 = Daudi; 9 = Raji; 10 = water control.
Fig 5:3. Heterogeneity in SSCP banding patterns produced by PCR products amplified from the EBV BamHI N region (upper panel) and K region (lower panel) in tongue scrapes of HIV-infected patients. Different patients are represented in different lanes. B. Comparison of BamHI N SSCP bands derived from blood (b) and tongue (t) specimens of 10 HIV-infected patients (nos. 1-10). C. Left panel: BamHI N SSCP banding patterns derived from whole saliva (s), tongue (t) and parotid saliva (p) of an HIV-infected patient (no. 11). Right panel: BamHI N SSCP banding patterns derived from whole saliva of 2 HIV-positive homosexual partners (patients 12 and 13) taken at 3-monthly intervals. Figures at bottom denote times of sampling (in months).
Fig 5:4. *BamHI K SSCP* banding patterns derived from tissue specimens taken from patients presumed not to be infected by HIV. 1 and 2 = lung (bronchogenic carcinoma); 3 and 4 = skin (squamous cell carcinoma); 5 = oral mucosa (mouth ulcer); 6 = oral mucosa (Sweet’s syndrome); 7 = post-nasal space mucosa (NPC).
Fig 5:5. SSCP banding patterns yielded by clones of BamHI N PCR products derived from an OHL (A) and NPC (B) biopsy specimen. In B, the middle band in the 4th lane from right is too faint to be reproduced in the figure.
Fig 6:1. $Z_2/Z_3$ genotyping of EBV amplified from the blood (b) and tongue (t) of study patients infected by HIV (HIV$^+$) and healthy controls (HIV$^-$). Figures at the top of the lanes indicate study numbers. The gel migratory positions of the BZLF-1 amplicon derived from the B95-8 virus (representing the $Z_2$ genotype) and AG876 virus (representing the $Z_3$) are shown. M=marker.
Fig 6.2. LMP/LMP<sub>del</sub> genotyping of EBV amplified from the blood (b) and tongue (t) of study patients infected by HIV (HIV<sup>+</sup>) and healthy controls (HIV<sup>-</sup>). The migratory position of the band from the B95-8 virus (representing the LMP genotype) is shown. Bands migrating to a position lower than this belongs to the LMP<sub>del</sub> genotype.
Fig 6:3. EBV types A and B in the tongue of HIV+ patients determined by gel electrophoresis of PCR products amplified from the EBNA-3C gene. AG876 represents type B and B95-8 represents type A virus.
Fig 6.4. SSCP analysis of the BZLF-1 amplicons derived from PBCs (b) and tongue (t) of patient 1 (upper panel) and patient 8 (lower panel).
APPENDIX

PLEASE ANSWER THE FOLLOWING QUESTIONS AS HONESTLY AS POSSIBLE

1.
I am willing to treat symptomless HIV-infected individuals  Yes  No
I am willing to treat ARC (AIDS Related Complex) patients  Yes  No
I am willing to treat patients with Acquired Immune Deficiency Syndrome  Yes  No

2. In General Practice:
   Undertaking the dental treatment of HIV-infected individuals:
   - is likely to be financially unrewarding  Yes  No
   - may result in loss of regular, non-infected patients  Yes  No
   - may cause the dentist to gain the reputation of being homosexual  Yes  No
   - may result in the loss of ancillary/nursing staff  Yes  No

3. I have an adequate knowledge of HIV infection that will ensure that I do not become infected with HIV.  Yes  No

4. Transmission of HIV is likely via: (please grade)

<table>
<thead>
<tr>
<th>Activity</th>
<th>Highly likely</th>
<th>Possibly</th>
<th>Most unlikely</th>
</tr>
</thead>
<tbody>
<tr>
<td>attending the hairdresser</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>social kissing</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>tears</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>sharing cups</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>vaginal intercourse</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>sharing razors</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>sharing toothbrushes</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

293
anal intercourse

oral sex

attending the chiropodist

breast feeding

blood transfusions

Highly likely
Possibly
Most unlikely

sharing needles

insect bites

ear piercing

swimming pools

mutual masturbation

wet kissing (french kissing)

5. In general dental practice HIV-infected patients should:

- have all oral care undertaken by his/her dentist        Yes  No
- should be refereed to a specialist dental unit (eg local dental school or hospital unit) for all their oral care    Yes  No
- should only be referred to specialist unit for the diagnosis and management of HIV-related oral problems    Yes  No

6. How would you assess the HIV status of a patient?

Please check the appropriate answer(s)

(a) Using a questionnaire filled in by the dentist

(b) Direct questioning of the patient by the dentist

(c) Direct questioning of the patient by the nurse or receptionist

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(d) Patient appearance/demeanor

(e) HIV testing the patient

(f) Determine if the patient has low number of lymphocytes (lymphocytes)

(g) I do not

(h) Other - please specify

7. All HIV-infected individuals develop AIDS

Condoms prevent transmission of HIV during sexual intercourse

All HIV-infected individuals will have a positive HIV test result

The General Dental Council has stated that HIV-infected dentists can no longer practice dentistry

Testing patients for HIV infection requires consent

Oral manifestations of HIV infection are common

Hepatitis B vaccination may protect against HIV

All HIV-infected individuals should be quarantined

8. Have you ever sustained an injury from a needle or instrument previously used in a patient?

If YES, please indicate approximately how many needlestick injuries you have received this year.

9. Please indicate your:

   Sex:

   Age:

   Professional qualifications whether student or graduate dentist

Students please indicate whether:
1st year
2nd year
3rd year
4th year
5th year

10 Age of first sexual intercourse?