HIV SALIVARY GLAND DISEASE:
A ROLE FOR VIRAL INFECTION

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A thesis submitted to the faculty of the University of North Carolina at Chapel Hill in partial fulfillment of the requirements for the degree of Master of Science in the Department of Oral and Maxillofacial Pathology School of Dentistry

Chapel Hill
2005

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ABSTRACT

Allan Dovigi: HIV SALIVARY GLAND DISEASE:
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(Under the Direction of Dr. Webster Cyriaque)

HIV-associated salivary gland disease (HIV SGD) is an AIDS defining condition associated with significant morbidity and lymphoma development in HIV-positive individuals. Understanding HIV SGD becomes increasingly important as the burden of HIV disease expands globally. The epidemiology of HIV SGD suggests the involvement of a viral opportunist in its pathogenesis. Based on this and on histologic correlates we hypothesized that HIV SGD is a manifestation of DNA tumor virus infection/reactivation during immunosuppression. Analysis of HIV SGD lesions determined that while herpesviral gene products were not consistently detected in HIV SGD, polyomavirus nucleic acids and antigens were detected. The subcellular localization of the viral-oncoprotein in HIV SGD was similar to that in a mouse model of polyomavirus-associated salivary gland disease. In HIV SGD the polyomavirus oncoprotein, T-antigen, was consistently co-localized with p53 implicating the deregulation of this tumor suppressor in the HIV SGD pathogenesis. Collectively, these studies underscore the potential for polyomaviruses to be key etiologic agents in HIV SGD development.

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Chapter 1

Literature Review

Salivary Gland Diseases

The studies that follow are significant because with the rising prevalence of HIV SGD it is increasingly important that we understand the pathogenesis of this disease.

There are a number of human salivary gland diseases including Sjogren Syndrome (an autoimmune disease), sialadenosis which is swelling of the parotid glands from chronic alcoholism or bulimia, sialadenitis from infections both viral (including mumps) and bacterial, and various neoplasms, some believed to be caused by DNA tumor viruses (Table 1). A large number of medications including cancer chemotherapeutics and radiation have direct effects on all the salivary glands producing sialadenitis, atrophy and xerostomia (Johnson, 1993; Narhi, 1999).

Benign Lymphoepithelial lesions are most commonly seen in patients with Sjogren Syndrome or HIV patients. These lesions are characterized histologically by two components seen in the parenchyma of the parotid gland or other salivary glands: epimyoeplithelial islands and an extensive lymphoid infiltrate. This is associated with enlargement of the parotid gland, xerostomia and the development of lymphoepithelial cysts in the affected gland. Patients with these diseases are at an increased risk of developing non-Hodgkin lymphomas (40 times that of the general population).

The majority of low-grade B cell lymphomas that arise in the setting of benign lymphoepithelial lesions are mucosa associated lymphoid tissue lymphomas (MALT), renamed Marginal Zone B cell lymphoma in the REAL classification (Bridges, 1989).

Sjogrens syndrome is a relatively common disease characterized by autoimmunity against the salivary and lacrimal glands. It is exceeded only by rheumatoid arthritis in occurrence and probably often goes undiagnosed as patients fail to report dry mouth and dry eyes as symptoms. In addition to benign lymphoepithelial lesions, Sjogren Syndrome is associated with the detection of autoantibodies (anti-Ro and anti-La as well as ANAs) and with infectious agents such as EBV, Hepatitis C and Human T-cell leukemia virus (James, 2001).

Sialadenosis presents as a painless recurrent swelling of the parotids. The peak incidence is in the fifth and sixth decades and there is no gender predilection. Microscopically the glands appear normal other than hyperplasia of the acinar cells (up to 2-3 times the regular size of these cells). If the disease is chronic there is eventual atrophy of the parenchyma and fatty replacement. These changes can occur in response to medications or hormonal imbalances. Other causes are diabetes, cirrhosis and bulimia electrolyte imbalances (Coleman, 1998).

Mumps (viral sialadenitis) has been associated with several viruses: herpes, influenza A, parainfluenza, cytomegalovirus and adenovirus. Paramyxovirus is the best known of the sialoadenotropic viruses. Clinically it is a self limiting disease that affects mainly children.

One or both of the parotid glands becomes painful and swollen. Pathologic features include: dense interstitial lymphoplasmacytic infiltrates, acinar cell vacuolization and ductal ectasia (Seifert, 1986).

There are a number of salivary gland tumors both benign and malignant that have been associated with a viral etiology. Simian virus 40 (SV40) sequences for the large T-antigen oncoprotein were investigated in DNA samples from human pleomorphic adenomas of parotid glands. Specific SV40 sequences were detected, by PCR and filter hybridization in human pleomorphic adenoma specimens. None of the DNA samples from normal salivary gland tissues was SV40-positive. Detection of SV40 sequences and T antigen expression in human pleomorphic adenomas suggests that this oncogenic virus may play a role as a cofactor in the onset and progression of this benign neoplasm. These Polyomaviral antigens have also been detected in a number of other neoplasms including CNS tumors (Martinelli, 2002).

Salivary Gland Disease	Histologic Characteristics	Etiology
Sjogren Syndrome	Lymphocytic infiltrates Sclerosis of gland	Unknown possibly Autoimmune, viral
Sialadenosis	Increased acinar cell size Increased number of zymogen granules in cells	Bulimia Alcoholism Diabetes Endocrine
Infectious	Interstitial lymphoplasmacytic infiltrates Acinar cell vacuolization Ductal ectasia	Herpes Influenza A CMV Adenovirus Paramyxovirus
Neoplasms	Proliferation of salivary gland parenchyma Proliferation of lymphoid component	Viruses DNA mutation radiation

Table 1. Salivary Gland Diseases and their basic histology and etiology

DNA Tumor Viruses and Their Associated Diseases

There are a number of families of DNA tumor viruses including, Papovaviruses, Adenoviruses, Herpesviruses and Hepadnaviridae. These viruses transform the cells they infect changing the biologic functions of the cell by regulating the cell with their viral oncogenes. This confers the cell with certain properties characteristic of neoplasia. These changes can result from integration of the viral genome into the host cell genome.

Herpesviruses

The Herpesviruses consist of a family of 8 known human Herpesviruses that are assigned to 3 subfamilies; alpha that includes herpes simplex 1 and 2 and Varicella zoster virus, beta (including human cytomegalovirus) and gamma (which includes both Epstein Barr virus (EBV) and Kaposi Sarcoma associated Herpesvirus). EBV, has been associated with Burkitt lymphoma and has been detected in as high as 30% of patients with Hodgkin lymphoma (Meyer RM et al, 2004). EBV also causes infectious mononucleosis (Fafi-Kremersis, 2005). Human cytomegalovirus is frequently associated with Kaposi sarcoma but this is now thought to be caused by a newly-discovered herpesvirus, human herpes virus 8 (HHV8) (Viejo-Borbolla, 2003). HHV8 or KSHV has been detected in 100% of KS tumors (Mendez, 1998). Herpes simplex II virus was associated in epidemiological studies with cervical cancer (Yang, 2004). Now the evidence for papillomavirus (a Papovavirus) being the causative agent of cervical cancer is far better.

Papovaviruses

The family name Papovavirus comes from the 3 prototypical members of PAPOVAVIRIDAE, Papillomavirus (PA), Polyomavirus (PO) and Vacuolating Agent Virus (VA) now called Simian Virus 40 (SV40). The Papovaviruses includes the human papillomas viruses. Papillomaviruses can cause abnormal tissue growth (genital warts) and other changes to cells. Infection with certain types of HPV (16, 18) may increase the risk of developing some types of cancer. They may also cause cancer in the oropharynx including the base of the tongue, soft palate and tonsils. High risk types 16 and 18 are known to cause squamous cell carcinoma (Liang Jin, 1999).

Polyomaviruses express 6 genes: Large T, Middle T, Small T, VP-1, VP-2 and VP-3. These are small DNA tumor viruses whose transforming properties are encoded by the large, middle and small T antigens (Tag), each of which has separate, complementary activities. Tag acts mainly by blocking the functions of p53 and RB tumor suppressor proteins, as well as by inducing chromosomal aberrations in the host cell (Welcker, 2005). Adenoviruses are highly oncogenic in animals but have not been associated with human cancers. Adenoviruses and Polyomaviruses seem to cause cell transformation in a similar manner as Adenovirus E1A and E1B also bind to the tumor suppressors p53 and RB to result in the genesis of transformed cellular phenotypes (Shay, 1991). Polyomaviruses, (the BK and JC subtypes) are known to cause kidney and colon tumors and have been detected in pleomorphic adenomas (Martinelli, 2002). 9.7% of children tested using PCR from the highly conserved T antigen region of the viral genome had polyomavirus in their urine which included BK, JC and SV40 (Butel, 2005). Human Polyomaviral strain BK has been detected in renal disease and is shed in urine (Vanchiere, 2005) Seroprevalence studies show that most primary infections with BK and JC occur in the first and second decades of life. Little is know about the transmission of these agents and the life long consequences of infection. Strain JC has been detected in association with brain tumors. Progressive multifocal leukoencephalopathy is a viral encephalitis caused by the JC virus. The virus preferentially infects oligodendrocytes and demyelination occurs. This disease is almost always seen in immunosupressed individuals. About 65% of the normal population have been exposed to JC by the age of 14 years (Chaisson, 1990).

Others have shown primary central nervous system lymphomas expressing polyoma JC virus genome (Del Valle, 2004).

Simianvirus 40 (SV40) is a monkey virus strain that was introduced in the human population by contaminated poliovaccines, produced in SV40-infected monkey cells, between 1955 and 1963. SV40 was detected in stocks of the Sabin poliovirus vaccine and in an adenovirus vaccine as well as the Salk polio vaccine. Vaccine administration resulted in the potential exposure of 100 million people to the virus (Shah, 1976). Epidemiological evidence now suggests that SV40 may be contagiously transmitted in humans by horizontal infection, independent of the earlier administration of SV40-contaminated poliovaccines. There is molecular evidence for SV40 infection in children born after vaccine administration (Butel, 1999). Butel et. al. detected BK and Simian Virus 40 in the urine of healthy children suggesting the ubiquity of infection early in life implicating that these viruses are endemic in the human population. There is serological evidence of SV40 infection in both HIV negative 12.0% and HIV infected adults 16.1%. (Jafar, 1998). Vaccinated individuals showed no increase in the development of cancer in epidemiologic studies (Fraumeni, 1963). Vaccinated immunocompetent individuals may have far lesser propensity for the development of cancer than others with defective immunity such as HIV/AIDS.

SV40 virus was shown to infect humans and cause tumors in experimental animals. SV40 sequences and expression of the large T antigen oncoprotein has been detected in pleomorphic adenomas of the parotid gland (Martinelli, 2002). SV40 sequences, SV40 antigen, and anti-SV40 antibodies in patients with tumors of the parotid gland have been demonstrated in oromaxillofacial tumors.

SV40 antigen was detected in malignant tumors that developed in the head and neck region (Stoian, 1987). SV40 has also been detected in non-Hodgkin lymphoma by PCR, sequence and Southern blot analysis (David, 2001; Shivapurkar, 2002; Vilchez, 2002; Nakatsuka, 2003). Previous studies have shown that Polyomaviral DNA has been detected in a number of tumors including benign salivary gland tumors and various CNS tumors (Shah, 1976; Butel, 1999).

SV40 Tag has been shown to induce tumor formation in slowly dividing epithelial cells of the lung and kidney (Choi., 1988). Acinar cells of the salivary glands are likely to fall into this category, resulting in the production of hyperplasias rather than tumor in most cases.

Opportunistic infections in the oral cavity in AIDS

The prevalence of the various types of oral cavity infections in HIV patients has changed recently. While Kaposi sarcoma and oral hairy leukoplakia have decreased, the incidence of HIV SGD (a suspected viral etiology) is increasing. In an HIV infected North Carolina population, HIV SGD has gone from 1.8% to 5%. (Patton, 1998), this increase has also been reported in other populations. (Greenspan, 1992)

There are several opportunistic infections that occur in the mouth in HIV patients. They include: Kaposi sarcoma, oral hairy leukoplakia, condyloma, thrush, herpes, aphthous ulcers and HIV SGD. Kaposi sarcoma is a malignancy associated with the HHV8. It is a malignancy of the blood vessels in the connective tissues which become infected when there is a breach in the mucosa. Oral hairy leukoplakia is an EBV infection causing hyperplasia of epithelium on the lateral borders of the tongue resulting in a hairy white appearance to the epithelium in this area (Niedobitek, 1992).

Condyloma accuminatum is an epithelial infection by the papillomavirus resulting in an excess proliferation of epithelial tissue resulting in outgrowths of cauliflower like lesions from the mucosa of the mouth (Aboulafia, 2002). Thrush is a primary infection by a fungal agent known as Candida. It colonizes the superficial layers of the mucosa and can appear as a white curd that is easily wiped off or as erythematous areas (Aboulafia, 2002).

Herpes in AIDS patients can occur anywhere in the mouth unlike herpes in immunocompetent individuals where it is localized to attached gingival tissues and the lips. These lesions can coalesce and become very large and painful. Aphthous ulcers are another painful lesion of unknown etiology, but a viral one is suspected. These are large ulcerations that occur in the mouth and are very painful. They can be associated with fever and usually last 10-14 days but in immunocompromised individuals can last months and cause scarring of the affected tissues.

Finally HIV SGD is a relatively recent condition recognized by enlargement of the parotid glands and complaints of xerostomia in the absence of xerostomic agents. Other than esthetic concerns HIV SGD results in all the morbidity associated with xerostomia including oral thrush, caries, periodontal disease and ultimately loss of teeth. This can be the presenting symptom in children with AIDS.

HIV SGD :

In 1986, parotid gland swelling was described by Rubinstein in pediatric AIDS patients (Rubinstein, 1986).

In 1987 Ulirsch et al. made the seminal observation that a Sjogren syndrome-like illness was associated with the acquired immunodeficiency syndrome-related complex. On the basis of immunohistologic studies, it was concluded that the increased epithelial metaplastic cellular component involved in the immune response might represent an exuberant reaction to HIV infection (Poletti, 1988) or that immune dysregulation may manifest itself as autoimmune reactivity (Calabrese, 1988).

The tie to malignancy was initially established in 1988 when a series of HIV-associated lymphatic lesions originating in salivary gland were surgically excised. Six cases of lymphadenitis and three cases of lymphoma, all originating in salivary gland lymph nodes were seen. They showed histologic lesions known to occur in association with AIDS (Ioachim, 1988).

An increase in the prevalence of HIV SGD concurrent with the inception of highly active antiretroviral therapy (HAART) in the AIDS population was reported by multiple groups in the mid to late 1990's (Patton, 2000). HAART may act as an effect modifier and those patients who are in the initial stages of HAART therapy and still have relatively high viral loads are at the greatest risk of developing HIV SGD. Upon the initiation of HAART, there is a HAART-associated immune recovery that may lead to activation of opportunistic infections that otherwise may have been clinically silent.

This phenomenon has been described in association with the development of mycobacterium avium complex (MAC) and HCMV retinitis.

HIV SGD is an AIDS defining illness in children. HIV SGD is often a presenting symptom in children with AIDS and it is increasing in this group.

HIV SGD is characterized by enlargement of the parotid gland and/or a complaint of xerostomia in the absence of xerostomic agents or diseases, lymphoepithelial cysts and lymphoma. Xerostomia predisposes to rampant tooth decay, oral candidiasis and progressive periodontal disease (Greenspan, 1992). Patients with HIV infection often complain of mouth dryness (xerostomia) (Navazesh, 2000; Younai, 2001) and it is suggested these effects are not due solely to the xerostomic producing drugs these patients are on but may also be due to the HIV infection.

Histologically HIV SGD is characterized by hyperplastic intraparotid lymph nodes and/or lymphocytic infiltrates within the salivary gland tissue. Long Standing HIV SGD is associated with development of lymphoepithelial cysts. These cysts are a local manifestation of long standing lymphadenopathy and are associated with diffuse infiltrative lymphocytosis syndrome (Williams, 1998). These features are also seen in minor salivary glands including infiltration of CD-8 T cells (Schoidt, 1989). Other studies showed a predilection for lymphoepithelial cysts in the HIV positive population (Ryan, 1985; Shaha, 1993). These are generally benign but have been associated with MALT lymphomas and can present an esthetic problem in this group of patients. Lymphoma results in significant morbidity and mortality in this group of patients.

Both the etiology and pathogenesis of HIV SGD are unknown. We hypothesize in Chapter 2 that the data showing different rates of HIV SGD in children (20-47%) versus adults (1%) may indicate a primary infection with an infectious agent versus residual immunity. The incidence of lymphoma in this population (1-2%) is also suggestive of an oncogenic etiology.

Further evidence supporting Polyoma virus as an etiologic agent are: 1) mice infected with polyomavirus develop enlarged parotid glands and a percentage of the mice develop malignancy, 2) SV40 sequences and expression of the large Tag oncoprotein has been detected in pleomorphic adenomas of the parotid gland (Martinelli, 2002). It has also been detected in Non-Hodgkin lymphoma by PCR, sequence and Southern blot analysis (David, 2001; Shivapurkar, 2002; Vilchez, 2002, Nakatsuka, 2003), 3) BK has been detected in glandular tumors such as kidney tumors and tumors of the prostate gland. Despite the 19 year history of the disease, we are still at a loss as to the cause of HIV SGD.

Animal Model of Salivary Gland Disease

Viral infection is also associated with the development of Salivary gland disease in experimental animals. Athymic nude mice infected with mouse polyomavirus were found to develop a wasting disease accompanied by parotid sialoadenitis with intranuclear inclusion bodies in ductal and acinar epithelial cells. Intranuclear and cytoplasmic inclusions of the parotid epithelium were found to express polyomavirus antigens (Ward, 1984).

Salivary epitheliomas induced by injection of neonatal mouse with mouse polyomavirus were infiltrated with immature T lymphocytes (Harrod, 1990). This infiltration is reminiscent of HIV SGD.

These infiltrates were also described in tumors that arose at the site of inoculation in newborn guinea pigs inoculated with SE polyomavirus (Eddy, 1960). These guinea pigs also develop salivary gland enlargement.

Transgenic mice provide useful model systems to examine the molecular basis of salivary gland tumorigenesis (Breuer, 1991; Furth, 1998). Conditional expression of Tag induces transcription of a tet-op-SV40 Tag gene in striated ductal cells of the salivary gland. Mice develop ductal hyperplasia, at 4 months of age and hyperplasias present at 7 months of age persisted. In Smgb Tag mice, submandibular gland adenocarcinomas of intercalated duct origin developed in more than 50 % of male mice by 12 months of age (Dardick, 2000).

Significance

The studies that follow are significant because with the rising prevalence of HIV SGD it is increasingly important that we understand the pathogenesis of this disease. The morbidity associated with this disease results from xerostomia and its effects on the oral cavity. The effects of xerostomia can be quite severe and include rampant tooth decay and periodontal disease resulting in tooth loss or loss of the entire dentition. Oral infections including Candida, altered taste and speech impairment are also seen in patients with xerostomia (Greenspan, 1992). Repeated oral fungal infections and the associated effects of these infections, lymphoepithelial cysts and the associated esthetic concerns and lymphoma (and associated mortality rates), are all serious concerns with this disease. Determination of an etiologic agent and cellular pathways that are altered may allow us to target and implement appropriate interventions that would decrease the morbidity associated with HIV SGD.

References:

- Aboulafia DM.** Condyloma acuminatum presenting as a dorsal tongue lesion in a patient with AIDS.
AIDS Read. 2002 ;12(4):165-7, 172-3
- Breuer M** The use of transgenic mice in unraveling the multistep process of tumorigenesis.
Radiat Environ Biophys. 1991;30(3):181-3
- Bridges AJ, England DM.** Benign lymphoepithelial lesion: relationship to Sjogren's syndrome and evolving malignant lymphoma.
Semin Arthritis Rheum. 1989 ;19(3):201-8
- Calabrese LH.** Autoimmune manifestations of human immunodeficiency virus (HIV) infection.
Clin Lab Med. 1988 ;8(2):269-79
- Chaisson RE, Griffin DE.** Progressive multifocal leukoencephalopathy in AIDS
JAMA. 1990 ;264(1):79-82
- Choi YW, Lee IC, Ross SR** Requirement for the simian virus 40 small tumor antigen in tumorigenesis in transgenic mice.
Mol Cell Biol. 1988 ;8(8):3382-90.
- Coleman H, Altini M, Nayler S, Richards A.** Sialadenosis: a presenting sign in bulimia
Head Neck. 1998 ;20(8):758-62
- Dardick I, Ho J, Paulus M, Mellon PL, Mirels L** Submandibular gland adenocarcinoma of intercalated duct origin in SmgB-Tag mice.
Lab Invest. 2000 ;80(11):1657-70
- David H, Mendoza S, Konishi T, Miller CW.** Simian virus 40 is present in human lymphomas and normal blood.
Cancer Lett. 2001 162(1):57-64
- Del Valle L, Enam S, Lara C, Miklosy J, Khalili K, Gordon J.** Primary central nervous system lymphoma expressing the human neurotropic polyomavirus, JC virus genome.
J Virol. 2004 ;78(7):3462-9
- Eddy BE, Borman GS, Kirschstein RL, Touchette RH** Neoplasms in guinea pigs infected with SE polyoma virus
J Infect Dis. 1960 ;107:361-8

- Fafi-Kremer S, Morand P, Brion JP, Pavese P, Baccard M, Germe R, Genoulaz O, Nicod S, Jolivet M, Ruigrok RW, Stahl JP, Seigneurin JM.** Long-term shedding of infectious epstein-barr virus after infectious mononucleosis.
J Infect Dis. 2005 ;191(6):985-9. Epub 2005 Feb 7
- Finch CJ, Butel JS.** Association between simian virus 40 and non-Hodgkin lymphoma.
Lancet. 2002 ;359(9309):817-23.
- Fraumeni JF Jr, Ederer F, Miller RW** An evaluation of the carcinogenicity of simian virus 40 in man
JAMA. 1963 ;185:713-8.
- Furth PA, Li M, Hennighausen L** Studying development of disease through temporally controlled gene expression in the salivary gland
Ann N Y Acad Sci. 1998 ;842:181-7
- Gnepp DR** Diagnostic Surgical Pathology of the Head and Neck
2001 W.B. Saunders Company 327-335
- Greenspan D, Komaroff E, Redford M, Phelan JA, Navazesh M, Alves ME, Kamrath H, Mulligan R, Barr CE, Greenspan JS.** Oral mucosal lesions and HIV viral load in the Women's Interagency HIV Study (WIHS).
J Acquir Immune Defic Syndr. 2000 ;25(1):44-50
- Greenspan D, Greenspan JS** Significance of oral hairy leukoplakia
Oral Surg Oral Med Oral Pathol. 1992 ;73(2):151-4
- Harrod FA, Kettman JR.** Transplantable polyoma virus-induced epitheliomas harbor immature T lymphocytes
Cell Immunol. 1990 ;125(1):29-41
- Ioachim HL, Ryan JR, Blaugrund SM.** Salivary gland lymph nodes. The site of lymphadenopathies and lymphomas associated with human immunodeficiency virus infection.
Arch Pathol Lab Med. 1988;112(12):1224-8
- Jafar S, Rodriguez-Barradas M, Graham DY, Butel JS.** Serological evidence of SV40 infections in HIV-infected and HIV-negative adults.
J Med Virol. 1998 ;54(4):276-84.
- James JA, Harley JB, Scofield HR** Role of viruses in systemic lupus erythematosus and Sjogrens syndrome
Current Opinion in Rheumatology 2001, 13:370-376

- Johnson JT, Ferretti GA, Nethery WJ, Valdez IH, Fox PC, Ng D, Muscoplat CC, Gallagher SC.** Oral pilocarpine for post-irradiation xerostomia in patients with head and neck cancer
N Engl J Med. 1993 ;329(6):390-5.
- Lednicky JA, Butel JS.** Polyomaviruses and human tumors: a brief review of current concepts and interpretations.
Front Biosci. 1999 ;4:D153-64
- Jin L, Qi M, Chen D, Anderson A, Yang G, Arbeit JM, Auborn KJ,** Indole-3-Carbinol Prevents Cervical Cancer in Human Papilloma Virus Type 16 (HPV⁺) Transgenic Mice
Cancer research 59, 3991-3997, 1999
- Martinelli M, Martini F, Rinaldi E, Caramanico L, Magri E, Grandi E, Carinci F, Pastore A, Tognon M.** Simian virus 40 sequences and expression of the viral large T antigen oncoprotein in human Pleomorphic adenomas of parotid glands.
Am J Pathol 2002 ;161(4):1127-33
- McKaig RG, Patton LL, Thomas JC, Strauss RP, Slade GD, Beck JD** Factors associated with periodontitis in an HIV-infected southeast USA study
Oral Dis. 2000 ;6(3):158-65
- Mendez JC, Procop GW, Espy MJ, Paya CV, Smith TF.** Detection and semiquantitative analysis of human herpesvirus 8 DNA in specimens from patients with Kaposi's sarcoma.
J Clin Microbiol. 1998 ;36(8):2220-2
- Meyer RM, Ambinder RF, Stroobants S.** Hodgkin's lymphoma: evolving concepts with implications for practice.
Hematology (Am Soc Hematol Educ Program). 2004;:184-202
- Nakatsuka S, Liu A, Dong Z, Nomura S, Takakuwa T, Miyazato H, Aozasa K;** Osaka Lymphoma Study Group. Simian virus 40 sequences in malignant lymphomas in Japan
Cancer Res. 2003 ;63(22):7606-8.
- Narhi TO, Meurman JH, Ainamo A** Xerostomia and hyposalivation: causes, consequences and treatment in the elderly
Drugs Aging. 1999 ;15(2):103-16
- Niedobitek G, Young LS, Lau R, Brooks L, Greenspan D, Greenspan JS, Rickinson AB** Epstein Barr virus infection in oral hairy leukoplakia: virus replication in the absence of a detectable latent phase
J Gen Virol. 1991 ;72 (Pt 12):3035-46

- Neville, Damm, Allen, Bouquot** Oral and Maxillofacial Pathology
Second Edition 2002 W.B. Saunders Company 398-405, 233-234
- Patton LL, McKaig RG, Strauss RP, Eron JJ Jr.** Oral manifestations of HIV in a
southeast USA population.
Oral Dis. 1998 ;4(3):164-9
- Poletti A, Manconi R, Volpe R, Carbone A.** Study of AIDS related
lymphadenopathy in the intraparotid and perisubmaxillary gland lymph nodes.
J Oral Pathol. 1988 ;17(4):164-7.
- Robbins** Pathologic Basis of Infectious Disease
1999 W.B. Saunders Company pg 1321
- Rubinstein A, Morecki R, Silverman B, Charytan M, Krieger BZ, Andiman W,
Ziprkowski MN, Goldman H.** Pulmonary disease in children with acquired immune
deficiency syndrome and AIDS-related complex.
J Pediatr. 1986 ;108(4):498-503.
- Ryan JR, Ioachim HL, Marmer J, Loubeau JM** Acquired immune deficiency
syndrome--related lymphadenopathies presenting in the salivary gland lymph
nodes.
Arch Otolaryngol. 1985 ;111(8):554-6.
- Schiodt M, Dodd CL, Greenspan D, Daniels TE, Chernoff D, Hollander H,
Wara D, Greenspan JS.** Natural history of HIV-associated salivary gland disease.
Oral Surg Oral Med Oral Pathol. 1992 ; 74(3):326-31.
- Schiodt M, Greenspan D, Levy JA, Nelson JA, Chernoff D, Hollander H,
Greenspan JS.** Does HIV cause salivary gland disease?
AIDS. 1989 Dec;3(12):819-22.
- Seifert G, Miehlik A, Haubrich J, Chilla R,** Diseases of the Salivary Glands,
Diagnosis, Treatment, Facial Nerve Surgery
Stuttgart: Gorg Thieme Verlag, 1986: 110-163
- Shah K, Nathanson N** Human exposure to SV40: review and comment
Am J Epidemiol. 1976 ;103(1):1-12
- Shaha AR, DiMaio T, Webber C, Thelmo W, Jaffe BM.** Benign lymphoepithelial
lesions of the parotid.
Am J Surg. 1993 ;166(4):403-6

- Shay JW, Pereira-Smith OM, Wright WE** A role for both RB and p53 in the regulation of human cellular senescence
Exp Cell Res. 1991 ;196(1):33-9
- Shivapurkar N, Harada K, Reddy J, Scheuermann RH, Xu Y, McKenna RW, Milchgrub S, Kroft SH, Feng Z, Gazdar AF.** Presence of simian virus 40 DNA sequences in human lymphomas.
Lancet. 2002 Mar 9;359(9309):851-2.
- Stoian M, Zaharia O, Suru M, Constantinescu E, Goldstein I, Nastac E.** Possible relation between viruses and oromaxillofacial tumors.Demonstration of hemagglutination-inhibiting anti-BK virus antibodies in patients with tumors of the parotid gland
Virologie. 1987 Jan-Mar;38(1):53-60.
- Strickler HD, Rosenberg PS, Devesa SS, Hertel J, Fraumeni JF Jr, Goedert JJ.** Contamination of poliovirus vaccines with simian virus 40 (1955-1963) and subsequent cancer rates.
JAMA. 1998 Jan 28;279(4):292-5.
- Ulirsch RC, Jaffe ES** Sjogren's syndrome-like illness associated with the acquired immunodeficiency syndrome-related complex
Hum Pathol. 1987 Oct;18(10):1063-8.
- Vanchiere JA, White ZS, Butel JS.** Detection of BK virus and simian virus 40 in the urine of healthy children.
J Med Virol. 2005 Mar;75(3):447-54
- Viejo-Borbolla A, Schulz TF.** Kaposi's sarcoma-associated herpesvirus (KSHV/HHV8): key aspects of epidemiology and pathogenesis
AIDS Rev. 2003 Oct-Dec;5(4):222-9
- Vilchez RA, Madden CR, Kozinetz CA, Halvorson SJ, White ZS, Jorgensen JL,** Association between simian virus 40 and non-Hodgkin lymphoma.
Lancet. 2002 Mar 9;359(9309):817-23.
- Ward JM, Lock A, Collins MJ Jr, Gonda MA, Reynolds CW** Papovaviral sialoadenitis in athymic nude rats
Lab Anim. 1984 Jan;18(1):84-9
- Webster-Cyriaque J.** Development of Kaposi's sarcoma in a surgical wound.
N Engl J Med. 2002 Apr 18;346(16):1207-10.
- Welcker M, Clurman BE.** The SV40 large T antigen contains a decoy phosphodegron that mediates its interactions with Fbw7/hCdc4.
Biol Chem. 2005 Mar 4;280(9):7654-8. Epub 2004 Dec 20

Williams FM, Cohen PR, Jumshyd J, Reveille JD. Prevalence of the diffuse infiltrative lymphocytosis syndrome among human immunodeficiency virus type 1-positive outpatients
Arthritis Rheum. 1998 May; 41(5): 863-8

Yang YY, Koh LW, Tsai JH, Tsai CH, Wong EF, Lin SJ, Yang CC. Correlation of viral factors with cervical cancer in Taiwan
J Microbiol Immunol Infect. 2004 Oct;37(5):282-7

Younai FS, Marcus M, Freed JR, Coulter ID, Cunningham W, Der-Martirosian C, Guzman-Bercerra N, Shapiro M. Self-reported oral dryness and HIV disease in a national sample of patients receiving medical care.
Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 2001 Dec;92(6):629-36.

Chapter 2

HIV Salivary Gland Disease: A Role for Viral Etiology

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INTRODUCTION

HIV SGD is an AIDS-defining illness in children and is increasing in this population (Patton, 2000). HIV SGD is characterized by enlargement of the parotid gland, lymphoepithelial cysts, lymphoma and a complaint of xerostomia (in the absence of xerostomic agents or diseases) (Greenspan, 1992). Xerostomia predisposes to rampant tooth decay, oral candidiasis and progressive periodontal disease. Histologically, HIV SGD is characterized by hyperplastic intraparotid lymph nodes and/or lymphatic infiltrates within the salivary gland tissue. Studies have shown these changes occur in both the major and minor salivary glands, including the labial salivary glands (Ulirsch et al, 1987).

The search for the etiology of HIV SGD has been inconclusive so far. Some argue that the disease is caused by autoimmune deregulation. Poletti argued that on the basis of immunohistologic studies, an exuberant reaction to HIV infection is characterized by an increased epithelial metaplastic cellular component (Poletti, 1988). Further immune dysregulation may manifest itself as autoimmune reactivity (Calabrese, 1988). In addition to autoimmune dysregulation others speculate a virus may play a role in disease development. Epstein Barr Virus and HIV have both been implicated in the disease (Rivera et al, 2002).

The epidemiology of HIV SGD suggests the involvement of a viral opportunist in its pathogenesis. Epidemiologic data showing different rates of HIV SGD in children (20-47%) versus adults (3-7.8%) may reflect primary viral infection versus reactivation, supporting an immunosuppressed state (DiGuiseppe, 1996). Interestingly, the incidence of salivary gland disease among HIV infected patients has increased following the introduction of Highly Active Antiretroviral Therapy (HAART) (Patton, 2000). HIV SGD becomes apparent shortly after induction of the drug regimen in HAART, reminiscent of other opportunistic infections reactivating and that are triggered by immune reconstitution. Opportunistic DNA viruses are frequently the etiologic agents of HIV associated oral lesions, and there has also been an increase in oral warts (Papillomavirus) in the era of HAART (Hille, 2002). Based on these epidemiologic correlates, we postulated that HIV SGD is a manifestation of a primary DNA tumor virus infection in children and reactivation in adults during immunosuppression.

The objective of the studies presented here was to seek candidate infectious viral etiologic agents for HIV SGD development, and to begin to determine their contribution to the disease. Two candidate tumor viral families are Herpesviridae and Polyomaviridae. Herpesviruses persistently infect salivary glands and are consistently detected in the saliva of HIV-infected individuals (reference XXX). While degenerate PCR did not detect a highly conserved region of the herpes viral genome, conserved polyomavirus genome was detected. In addition, no herpesviral gene products were detected, however, polyomavirus nucleic acids and antigens were detected. Further, the homologous polyomaviral oncoprotein expressed in mouse salivary gland disease displayed similar subcellular localization to the oncoproteins in HIV SGD tissue.

Collectively, these studies underscore the potential for polyomaviruses to be key etiologic agents in HIV SGD development.

METHODS

Patients, Animals and Sample Collection.

HIV positive patients were recruited from UNC Hospitals Dental Clinic or infectious disease clinic to participate in the IRB approved study (Study # 04-DENT-356 HIV SGD). Labial minor salivary gland biopsies were performed and tissues were frozen and embedded for sectioning. Transgenic mice were generated in an FVB background (Friend Virus B strain) (Charles River Laboratories, Wilmington Mass) expressing the MMTV long terminal repeat/PyV-mT. Controls were FVB wild type mice without the transgene.

DNA Isolation and Polymerase Chain Reaction. DNA was extracted by pulverizing frozen tissue and ultracentrifugation on a 4M guanidine isothiocyanate-cesium chloride step gradient as previously described (Webster-Cyriaque, 1998). Single-stranded DNA oligonucleotides were synthesized for PCR primers. PCR amplifications were performed with 150-200 ng of DNA template using primers specific for herpesviral terminase, polyomavirus Tag or GAPDH (control), with amplification proceeding for 38 cycles as previously described (Webster-Cyriaque, 1998).

Herpesvirus Degenerate PCR The published primers were designed to amplify a consensus sequence in the herpesviral DNA terminase gene (Hargis, 1999).

The following DNAs were amplified: 2 ng B958 genomic DNA (EBV), 2 ng HCMV-infected cells, and 20 ng BCBL-1 genomic DNA (KSHV) and 500 ng of each salivary gland DNA sample.

Polyomavirus Degenerate PCR Using published primers for nested PCR (van Gorder, 1999) we amplified polyomavirus genomes in the aforementioned salivary gland DNA.

Housekeeping gene In each case, the cellular gene GAPDH was amplified to control for DNA quality and loading.

Cloning and Sequencing

Products were TA-cloned and sequenced; a BLAST search was used to identify known sequences.

Immunofluorescence

Immunofluorescence was employed to identify the subcellular location of viral and cellular proteins in diseased tissue compared to normal controls. Frozen tissue sections were cut to 5 um thickness and placed on poly-L-lysine coated slides. Tissue sections and cell lines were fixed in a chilled 1:1 mixture of methanol and acetone, blocked with 20% normal goat serum, stained with primary antibody p53 polyclonal, p53 monoclonal, T-antigen (Tag) monoclonal, Tag polyclonal (Santa Cruz), then stained with fluorescein-conjugated isothiocyanate (FITC) goat anti-mouse IgG or lissamine rhodamine goat anti-rabbit IgG (Molecular Probes). Slides were mounted with Vectashield (Vector Laboratories) then subjected to confocal microscopy. Confocal images were overlaid using the image processing tool kit.

RESULTS

Degenerative PCR failed to detect herpesviral DNA. Using degenerative polymerase chain reaction, herpesviruses were assayed initially to determine their potential as etiologic agents of HIV SGD. While the highly conserved herpes terminase gene was detected in cell lines infected with EBV, CMV and KSHV, herpesviral DNA was not detected in 3/3 HIV SGD patients tested (Figure 1). Further, viral microarrays containing multiple open reading frames from each of the known human herpesviruses failed to detect herpes viral RNA in HIV SGD patients (data not shown). Finally, immunohistochemical studies did not detect EBV or KSHV viral proteins in HIV SGD (data not shown).

Figure 1. Herpes viral DNA was not detected in HIV SGD. The gel shows control cell lines infected with the herpesviruses (lanes 4-6) containing EBV (infected B958 cells), KSHV(infected BCBL1 cells) and CMV (infected HEL cells). The highly conserved terminase region of each control was readily amplified. Herpesviral DNA was not detected in HIV SGD by this assay (lanes 1, 2 and 3).

Figure 2. Polyoma virus mRNA is detected in HIV SGD. Two rounds of amplification of the Tag region were performed, yielding an expected amplification product of 174 bp in two patients with HIV SGD (lanes A & C) but not in the HIV positive patient without the disease (lane B). GAPDH amplification serves as a control for cellular DNA quality with an expected amplification product of 451bp.

Degenerative PCR detected polyomavirus DNA. Subsequently, the role of polyomaviruses in HIV SGD was investigated using degenerative PCR specific for the Tag region of polyomaviruses. Nested PCR detected a band of the expected size (174 base pairs) in two of two HIV SGD patients (Figure 2 lanes A and C), and not in an HIV patient without HIV SGD (Figure 2 lane B). These bands were then isolated from the gel, and were cloned into a PCR2.1 vector that did not contain polyomavirus sequences. Clones were then sequenced in order to confirm the presence of polyomavirus DNA in the disease (Figure 3). Amplification sequence was compared to known genomes by BLAST search, and was found to match the Tag regions of the polyomaviruses SV40, BK and JC.

SV40Strain777_440...	#1	TAGATTCCAACCTATGGAAGTGAATGGGAGCAGTGGTGGAATGCCTTTAATGAGGA
SGDMinusSecondary...	#1	TAGGTGCCAACCTATGGAAGTGAATGGGAGCAGTGGCGGAATGCCTTTAATGAGGA
SGDPlusSecondaryP...	#1	TAGGTGCCAACCTATGGAAGTGAATGGGAGCAGTGGTGGAATGCCTTTAATGAGGA
		
	#1	TAGGTGCCAACCTATGGAAGTGAATGGGAGCAGTGGTGGAATGCCTTTAATGAGGA
••	•	
SV40Strain777_44...	#60	AAACCTGTTTTGCTCAGAAGAAATGCCATCTAGTGATGATGAGGCTACTGCTGACTCTC
SGDMinusSecondar...	#60	AAACCTGTTTTGCTCAGAAGAAATGCCATCTAGTGATGATGAGGCTACTGCTGACTCTC
SGDPlusSecondary...	#60	AAACCTGTTTTGCTCAGAAGAAATGCCATCTAGTGATGATGAGGCTACTGCTGACTCTC
		
	#60	AAACCTGTTTTGCTCAGAAGAAATGCCATCTAGTGATGATGAGGCTACTGCTGACTCTC
SV40Strain777_4...	#119	AACATTCTACTCCTCCAAAAAGAAGAGAAAGGTAGAAGACCCCAAGGACTTTCC
SGDMinusSeconda...	#119	AACATTCTACTCCTCCAAAAAGAAGAGAAAGGTAGAAGACCCCTAAAGACTTTCCA
SGDPlusSecondar...	#119	AACATTCCACTCCTCCAAAAAGAAGAGAAAGGTAGAAGACCCCTAAAGACTTTCCA
		
	#119	AACATTCTACTCCTCCAAAAAGAAGAGAAAGGTAGAAGACCCCTAAAGACTTTCCA
•	••	

Figure 3. Sequence analysis of degenerate PCR products from the HIV SGD patients correlated with the highly conserved Tag region of SV40, BK and JC.

Immunofluorescence colocalized Tag and p53 in HIV SGD. Following polyomavirus nucleic acid detection, subsequent studies were performed, using immunofluorescence techniques, to identify and localize viral proteins within HIV SGD tissue. Labial minor salivary glands were harvested from 4 patients with HIV SGD and 2 HIV negative patients. Detection of polyomavirus Tag was assayed using antibodies targeted toward conserved epitopes that would detect Tag of SV40, BK or JC virus. Immunofluorescence, performed using Tag monoclonal antibodies, exhibited punctuate perinuclear staining in 4/4 HIV SGD patients, and lack of staining in 2/2 HIV negative individuals (Figure 4). A two tailed Fisher exact test that examines the strength of association between detection of Tag and presence of HIV SGD, detected a P value of 0.067 which trends toward statistical significance (Table 2). P53 specific polyclonal antibodies detected distinct intracellular perinuclear punctuate staining in the 4/4 HIV SGD patients, while this staining pattern was not detected in HIV-negative patients (Figure 4).

Confocal microscopy detected co-localization of Tag and p53 in HIV SGD. Tag specific monoclonal (green) antibody and p53 specific polyclonal (red) antibody exhibited punctuate perinuclear staining in HIV SGD (Figure 5). Superimposition of the two chromogens for Tag and p53 demonstrated co-localization of these antigens within salivary gland ductal cells in 4 of 4 of the HIV SGD subjects assayed. Tag and p53 was not detected in HIV SGD negative patients.

Detection of HIV p24 in HIV SGD. Others have detected HIV proteins within HIV SGD (Rivera, 2003). We assayed HIV p24 viral protein in our HIV SGD specimens. One patient tested was positive for cytoplasmic p24 (data not shown).

Figure 4. Perinuclear punctate staining pattern is observed in the HIV SGD patients for Tag monoclonal and p53 polyclonal antibodies.

Figure 5. Co-localization of Tag and p53 in HIV SGD: There is positive staining for Tag and p53 in the HIV SGD patients versus the HIV negative patients. The Tag and p53 chromogens are co-localized producing a yellow signal which is readily apparent in patients 1, 2 and 5. Patient 6 exhibits more background staining but the same pattern is discernable.

Tag was detected in mouse parotid gland hyperplasia. It is well established that expression of Tag causes salivary gland enlargement in mice. A transgenic mouse model of polyoma middle T expression (Ellies, 2003) expressing the antigen from the MMTV promoter was used to determine whether the localization of Tag in the mouse salivary gland disease has demonstrated similarities to the HIV SGD. Similar to HIV SGD, these transgenic mice exhibited atypical hyperplasia of the parotid tissue and lymphocytic infiltrates. Transgene expression was confirmed by Northern blot analysis. (data not shown). Parotid gland tissue from both wild type mice and from polyoma Tag transgenic mice was assayed for expression of Tag using immunofluorescence. A punctuate staining pattern for Tag, similar to that for HIV SGD, was detected in 3/3 transgenic mice, while 4/4 control mice did not show reactivity (Figure 6).

Figure 6. Punctate staining pattern is detected in MMTV PyT transgenic mice but not in Wild Type Mouse: The wild type mouse showed consistent lack of staining for the Tag. Both the negative controls and the polyclonal antibody show no staining other than some background staining.

	Patient	T ag Monoclonal	T ag Polyclonal	P 53 Monoclonal	P 53 Polyclonal	T ag & P 53	P 24
HIVSGD	1	+(2)	+(2)	+(2)	+(2)	+(2)	+
	2	+(3)	+(1)	+(1)	+(1)	+(1)	
	5	+(4)	+(1)	+(1)	+(1)	+(1)	
	6	+(1)	+(1)	+(1)	+(1)	+(1)	
HIV Negative	3	- (7)	-(2)	-(2)	-(2)	-(2)	-
	4	-(1)	-(1)	-(1)	-(1)	-(1)	
Transgenic Mouse	115		+(1)				
	117		+(1)				
	142		+(1)				
Wild Type Mouse	118		-(1)				
	119		-(1)				
	120		-(1)				
	121		-(1)				

Table 2. Consistent Detection of Tag and p53 in HIV SGD by Immunofluorescence: The HIV SGD positive patients consistently showed positive expression of Tag and p53 vs. the HIV negative control patients. Monoclonal and polyclonal antibodies for each protein were used. Similar results were seen in the transgenic vs. wild type mice. P24 was also detected in an HIV SGD patient. (#) indicates the number of tissue sections stained for each patient. Empty cells indicate test not performed.

Fisher Exact Test

		Tag Staining		
		positive	negative	
HIV SGD	4	0	4	
HIV Negative	0	2	2	
		4	2	6

Table 3. The association between Tag and HIV SGD trends towards statistical significance. 2 tailed Fisher exact test Tag monoclonal P value 0.067.

Discussion

Understanding viral and cellular determinants of disease is essential to understanding the pathogenesis of HIV SGD. Molecular guidelines for causation based on Koch's postulates have been established (Fredricks and Relman 1998). These have been used to justify Kaposi sarcoma-associated herpes virus (KSHV), an HIV-associated opportunistic infection, as the etiologic agent of Kaposi Sarcoma .

Koch's Postulates

- 1. The specific organism should be shown to be present in all cases of animals suffering from a specific disease but should not be found in healthy animals.*
- 2. The specific microorganism should be isolated from the diseased animal and grown in pure culture on artificial laboratory media.*
- 3. This freshly isolated microorganism, when inoculated into a healthy laboratory animal, should cause the same disease seen in the original animal.*
- 4. The microorganism should be re-isolated in pure culture from the experimental infection.*

This Fredricks-Relman model states:

- 1) The nucleic acid sequence belonging to a putative pathogen should be present in most cases of an infectious disease and preferentially within those sites known to be diseased.
- 2) Fewer or no nucleic acid sequences should occur in hosts and tissues without disease.

Based on this model, the objective of these studies was to determine whether known herpes or polyomaviruses contributed to the development of HIV SGD. We have provided evidence that a polyomavirus is involved in the pathogenesis of HIV SGD, that viral gene products belonging to polyomavirus were detected in most cases of HIV SGD and that these gene products were not detected in salivary gland tissues from hosts without the disease. Herpesvirus DNA was not detected in HIV SGD, herpesvirus transcripts were not detected by viral microarray, and EBV LMP1 or KSHV Lana protein were not detected by immunohistochemistry. While EBV could be detected by nested PCR (data not shown), lack of protein or transcripts do not support a role for it as an etiologic agent.

Others have shown that EBV, CMV and KSHV are not factors in the pathogenesis of HIV lymphoepithelial lesions (Chetty, 1999; Yen, 2004)). In contrast to our lack of detection of herpesviruses in HIV SGD, Rivera et al. have consistently detected EBV LMP 1 protein; however, the cytoplasmic membrane protein was only detected in the nucleus in these studies, suggesting possible cross-reactivity of the antibody with a nuclear protein. They also showed that HIV could infect salivary gland ductal cells in HIV-positive individuals. In our laboratory, HIV p24 antigen could be detected in the ductal epithelial cells of an HIV SGD patient, as well (data not shown). The ability to detect HIV within HIV SGD suggests that HIV co-infection may be important to the pathogenesis of the disease.

In this study, polyomavirus DNA was detected by degenerate PCR and was confirmed by sequence analysis of degenerate PCR products. Immunofluorescence studies of labial minor salivary gland tissue in HIV SGD patients were conclusive, showing consistent detection of a perinuclear punctuate staining pattern of Tag with both virus-specific monoclonal and polyclonal antibodies in the diseased patients. HIV negative patients were consistently negative (Table 2).

Previous investigators have demonstrated upregulation in polyomavirus infected cells, and binding of the large Tag to p53, altering the cell cycle and resulting in tumorogenesis (Welcker, 2005). We have shown that p53 is upregulated in HIV SGD, and have co-localized Tag with p53 in our diseased tissues. These findings suggest that not only is the Tag present in the diseased tissue, but that it is functional, and that it's binding to p53, which may disrupt downstream cellular targets contributing to pathogenesis. Further, parotid gland tissue from MMTV/T antigen transgenic mice showed positive staining for the Tag with the same punctate perinuclear staining pattern observed in HIV SGD tissue. Collectively, these results argue for the role of polyomavirus as an etiologic agent in the pathogenesis of HIV Salivary Gland Disease.

These results, taken together with the epidemiologic correlates of the disease, have led us to develop a model which postulates that during childhood, polyomavirus is acquired by primary infection (Figure 8). Subsequent to immune suppression, the child can develop HIV SGD and associated morbidity. A child who is not immunocompromised may undergo a subclinical infection. In adulthood, it is possible that either immune suppression-associated reactivation, or primary infection during immunosuppression results in HIV SGD and its associated morbidity.

Elucidating the cause and pathogenesis of this disease is a first step in the diagnosis and treatment of this emerging disease. These data support the hypothesis that polyomavirus plays a role in the etiology of HIV Salivary Gland Disease and that viral gene products disrupting cellular machinery are contributory to pathogenesis. Many of the known polyomavirus strains are ubiquitous in the population, infecting up to 80% of the population.

Future studies will be performed to identify and localize other polyomaviral antigens such as VP1, VP2 and VP3 within the infected tissue. Differential gene expression will be employed to identify modulation of host cellular pathways, and to determine how this virus is interacting with the host to produce the observed pathology.

Figure 7 Proposed Role for Infectious Agent: In childhood, immune suppression can lead to HIV SGD. In contrast, a healthy child with a polyomavirus infection does not develop HIV SGD. In adulthood, immune suppression reactivation of the virus can occur or primary infection may result in HIV SGD, with 1-2% progressing to lymphoma

References:

- Atkinson JC, Schiodt M, Robataille S, Greenspan D, Greenspan JS, Fox PC.** Salivary auto antibodies in HIV-associated salivary gland disease. J Oral Pathol Med. 1993 ;22(5):203-6.
- Bartek J, Vjtesek B, Grand RJ, Gallimore PH, Lane DP,** Cellular localization and T antigen binding of the retinoblastoma protein. Oncogene 1992, 7;101-108.
- Baumgarth N, Szubin R, Dolganov GM, Watnik MR, Greenspan D, DaCosta M, Palefsky JM, Jordan R , Roederer M, Greenspan JS.** Highly tissue substructure-specific effects of human papillomas virus in mucosa of HIV-infected patients revealed by laser-dissection microscopy-assisted gene expression profiling. Am J Pathol 2004, 165:707-718.
- Chang Y, Moore PS.** Kaposi's Sarcoma (KS)-associated herpesvirus and its role in KS. Infect Agents Dis. 1996 ; 5(4):215-22. Review.
- Chetty R, Vaithilingum M, Thejpal R.** Epstein Barr virus status and the histopathological changes of parotid gland lymphoid infiltrates in HIV-positive children. Pathology. 1999 ;31(4):413-7.
- Ellies LG,** PyV-mT-induced parotid gland hyperplasia as detected by altered lectin reactivity is not modulated by inducible nitric oxide deficiency. Archives of Oral Biology (2003) 48, 415-422.
- Favia G, Capodiferro S, Scivetti M, Lacatia MG, Filosa A, Lo Muzio L.** Multiple parotid lymphoepithelial cysts in patients with HIV-infection: report of two cases. Oral Diseases 2004, 10:151-154.
- Fredricks DN, Relman DA** Infectious agents and the etiology of chronic idiopathic diseases Curr Clin Top Infect Dis. 1998;18:180-200.
- de Fromentel CC, Viron A, Puvion E, May P.** SV-40 large T-antigen and transforming related protein p53 are associated in situ with nuclear RNP structures containing hnRNA of transformed cells. Exp Cell Res. 1986 ;164(1):35-48

- Hille JJ, Webster-Cyriaque J, Palefski JM, Raab-Traub N.** Mechanisms of expression of HHV8, EBV and HPV in selected HIV-associated oral lesions. Oral Dis. 2002;8 Suppl 2:161-8.
- Lin AL, Johnson DA, Stephan KT, Yeh CK.** Alteration in salivary function in early HIV infection. J Dent Res 2003, 82(9):719-124.
- Maglione JE, McGoldrick ET, Young LJ, Namba R, Gregg JP, Liu L, Moghanaki D, Ellies LG, Borowsky AD, Cardiff RD, MacLeod CL.** Polyoma virus middle T-induced mammary intraepithelial neoplasia outgrowths: Single origin, divergent evolution, and multiple outcomes. Mol Cancer Ther 2004;3(8). August 2004.
- Martinelli M, Martini F, Rinaldi E, Caramanico L, Magri E, Grandi E, Carinci F, Pastore A, Tognon M.** Simian virus 40 sequences and expression of the large T antigen oncoproteins in human pleomorphic adenomas of the parotid glands. American J. Pathol 2002, Vol 161, No 4; 1127-1133.
- McArthur CP, Africa CW, Castellani WJ, Luangjamekorn NJ, McLaughlin M, Subtil-DeOliveira A, Cobb C, Howard P, Gustafson S, Palmer D, Miranda RN** Salivary gland disease in HIV/AIDS and primary Sjogren's Syndrome: analysis of collagen 1 distribution and histopathology in American and African patients J Oral Pathol Med 2003; 32: 544-51
- Moore PS, Gao SJ, Dominguez G, Cesarman E, Lungu O, Knowles DM, Garber R, Pellett PE, McGeoch DJ.** Primary characterization of a herpesvirus agent associated with Kaposi sarcoma. J Virol. 1996 ; 70(1): 549-58. Erratum in: J Virol 1996 Dec; 70(12): 9083.
- Moore PS, Chang Y.** Detection of herpesvirus-like DNA sequences in Kaposi's sarcoma in patients with and without HIV infection. N Engl J Med. 1995 ;332(18): 1181-5.
- Morris TJ, Stanley EF.** A simple method for immunocytochemical staining with multiple rabbit polyclonal antibodies. Journal of Neuroscience Methods 127 (2003) 149-155
- Patton LL, McKaig RG, Strauss RP, Eron JJ Jr.** Oral manifestations of HIV in a southeast USA population. Oral Dis. 1998 ;4(3):164-9

Prochorec-Sobieszek M, Wagner T, Loukas M, Chwalinska -Sadowska H, Olesinska M. Histopathological and immunohistochemical analysis of lymphoid follicles in labial salivary glands in primary and secondary Sjogrens syndrome.
Med Sci Monit, 2004; 10(4): BR115-121.

Rivera H, Nikitakis NG, Castillo S, Siavash H, Papadimitriou JC, Sauk JJ. Histopathological analysis and demonstration of EBV and HIV p-24 antigen but not CMV expression in labial minor salivary glands of HIV patients affected by diffuse infiltrative lymphocytosis syndrome.
J Oral Pathol Med 2003; 32: 431-7.

Schiodt M, Dodd CL, Greenspan D, Daniels TE, Chernoff D, Hollander H, Wara D, Greenspan JS. Natural history of HIV-associated salivary gland disease.
Oral Surg Oral Med Oral Pathol. 1992 ; 74(3):326-31.

Schiodt M, Greenspan D, Levy JA, Nelson JA, Chernoff D, Hollander H, Greenspan JS. Does HIV cause salivary gland disease?
AIDS. 1989 ;3(12):819-22.

Schiodt M, Greenspan D, Daniels TE, Nelson J, Leggott PJ, Wara DW, Greenspan JS. Parotid enlargement and xerostomia associated with labial sialadenitis in HIV-infected patients.
J Autoimmun. 1989 ;2(4):415-25.

van Gorder MA, Della Pelle P, Henson JW, Sachs DH, Cosimi AB, Colvin RB Cynomolgus polyoma virus infection: a new member of the polyoma virus family causes interstitial nephritis, ureteritis, and enteritis in immunosuppressed cynomolgus monkeys.
Am J Pathol. 1999 Apr;154(4):1273-84

Wagner VE, Bushnell D, Passador L, Brooks AL, Iglewski BH. Microarray analysis of *Pseudomonas aeruginosa* quorum sensing regions: effects of growth phase and environment.
J Bacteriol. 2003 ; 185(7):2080-95.

Welcker M, Clurman BE. The SV40 large T antigen contains a decoy phosphodegron that mediates its interactions with Fbw7/hCdc4.
J Biol Chem. 2005 ;280(9):7654-8. Epub 2004 Dec 20

Williams FM, Cohen PR, Jumshyd J, Reveille JD. Prevalence of the diffuse infiltrative lymphocytosis syndrome among human immunodeficiency virus type 1-positive outpatients
Arthritis Rheum. 1998 ; 41(5): 863-8

Yen TL, Murr AH, Rabin J, Mhatre AN, Lalwani AK, Role of Cytomegalovirus, Epstein-Barr Virus, and Human Herpes Virus-8 in Benign Lymphoepithelial Cysts of the Parotid
Laryngoscope. 114(8):1500-1505, 2004