ORAL DISEASES

Oral Diseases (2011) 17, 258–264 doi:10.1111/j.1601-0825.2010.01733.x

www.wiley.com

ORIGINAL ARTICLE

PLUNC protein expression in major salivary glands of HIV-infected patients

AA da Silva[1], L Bingle[2], PM Speight[2], CD Bingle[3], T Mauad[4], LFF da Silva[4], PA Vargas[1]

[1]*Department of Oral Diagnosis, Oral Pathology Section, School of Dentistry of Piracicaba, UNICAMP, Piracicaba, Sao Paulo State, Brazil;* [2]*Department of Oral Pathology, School of Clinical Dentistry, University of Sheffield, Sheffield, UK;* [3]*Academic Unit of Respiratory Medicine, University of Sheffield Medical School, Royal Hallamshire Hospital, Sheffield, UK;* [4]*Laboratory of Pollution (UMOS), Department of Pathology, School of Medicine of São Paulo-USP, São Paulo-SP, Brazil*

OBJECTIVE: To analyse and compare the expression of Palate, Lung, and Nasal Epithelium Clone (PLUNC) proteins in salivary glands from patients with and without AIDS (control group) using autopsy material.

METHODS: We analysed the expression of PLUNCs using immunohistochemistry in parotid (*n* = 45), submandibular (*n* = 47) and sublingual gland (*n* = 37) samples of AIDS patients [30 with normal histology, 21 with mycobacteriosis, 14 with cytomegalovirus (CMV) infection, 30 with chronic non-specific sialadenitis, and 30 HIV-negative controls. *In situ* hybridization (ISH) for SPLUNC 2 in the HIV-negative group was performed.

RESULTS: SPLUNC 1 expression was detected in the mucous acini of submandibular and sublingual glands, and SPLUNC 2 were seen in the serous cells. LPLUNC 1 expression was only positive in the salivary ducts. There was a higher expression of SPLUNC 2 in AIDS patients with CMV infection and mycobacteriosis when compared with all other groups. The intensity of staining for SPLUNC 2 was greater around the lesions than the peripheral ones. ISH for SPLUNC 2 showed perinuclear positivity in the serous cells in all HIV-negative cases.

CONCLUSIONS: SPLUNC 1 and LPLUNC 1 proteins were similarly expressed in the salivary glands of AIDS patients and non-HIV patients. CMV infection and mycobacteriosis increase SPLUNC 2 expression in serous cells in the salivary gland of AIDS patients.

Oral Diseases (2011) **17**, 258–264

Keywords: PLUNC; HIV patients; salivary gland; immunohistochemisty

Correspondence: Dr Pablo Agustin Vargas, Oral Pathology Section, Oral Diagnosis Department, School of Dentistry of Piracicaba-UNICAMP, Av. Limeira 901, Caixa Postal 52, CEP:13414-903, Piracicaba-SP, Brazil. Tel: +55 19 21065319, Fax: +55 19 21065218, E-mail: pavargas@fop.unicamp.br
Received 9 April 2010; revised 28 May 2010; accepted 15 June 2010

Introduction

The cumulative, global, number of cases of acquired immunodeficiency syndrome (AIDS) surpassed 33 million in 2009 (UNAIDS, 2009). Infections, neoplasms and inflammatory conditions associated with AIDS often involve the salivary glands and detection of these lesions is considered an important outcome measure contributing to staging and prognosis of the disease (Greenspan *et al*, 2000; León *et al*, 2009). Salivary gland infections associated with AIDS include tuberculosis (Singh *et al*, 1998; Vargas *et al*, 2003; Miziara *et al*, 2005), histoplasmosis (Raab *et al*, 1994; Hernandéz 2004; Joshi *et al*, 2006), mycobacteriosis (Elvira *et al*, 1998; Ohtomo *et al*, 2000; Vargas *et al*, 2003; De Faria *et al*, 2005; Rangel *et al*, 2005; León *et al*, 2009), cryptococcosis (Monteil *et al*, 1997; Vargas *et al*, 2003; León *et al*, 2009) and cytomegalovirus (CMV) (Wagner *et al*, 1996; Vargas *et al*, 2003; León *et al*, 2009), non-Hodgkin lymphoma and Kaposi's sarcoma are common malignancies in AIDS patients and inflammatory disorders include lymphoepithelial cysts (Vargas *et al*, 2003; León *et al*, 2009) and sialadenitis (Gomes *et al*, 2006).

The mucosal surface of the oral cavity is continually exposed to large, complex, commensal microbial populations, which elicit no significant response despite intimate contact. An effective innate immune system is of central importance in maintaining this host-microbe homeostasis and thus preventing infection. In particular, oral tissues appear to be naturally resistant to human immunodeficiency virus type 1 (HIV 1) infection (Lu and Jacobson, 2007; Rocha *et al*,2008; León *et al*, 2009).

Palate, Lung, and Nasal Epithelium Clone (PLUNC) was first described in the nasal epithelium of the mouse embryo and the trachea and bronchi of adult mouse lung (Weston *et al*, 1999). We have shown that human PLUNC belongs to a family of 10 genes located on chromosome 20q11 and that they are expressed in the upper airway, oro- and nasopharynx. We have also shown that PLUNC proteins demonstrate significant structural similarity to LPS binding protein (LBP) and

Bactericidal/Permeability-Increasing protein (BPI), two proteins, which are critical to the mediation of signals from bacterial lipopolysaccharide (LPS), a major microbial effector molecule present in cell walls of Gram-negative bacteria. Our structural data also indicates that PLUNC proteins can be subdivided into short (SPLUNC) and long (LPLUNC) proteins, with the 'short' proteins (around 25 kDa) having homology to the N-terminal domain of BPI/LBP and the 'long' proteins (around 40 kDa) having homology to both domains of BPI/LBP. In this terminology the prototypic protein originally identified as PLUNC becomes SPLUNC 1 and there are four 'short' proteins (1–4) and six 'long' proteins (1–6) (Bingle and Bingle, 2000; Bingle and Craven, 2002; Bingle *et al*, 2004, 2009). Due to the structural similarities of PLUNC proteins, BPI and LBP we have hypothesized that PLUNCs will play a role in innate immunity, however, recent studies have suggested that SPLUNC 1 may also act as a surfactant and play a role in airway surface liquid volume regulation (Yuan *et al*, 1997).

To date there are no reports in the literature investigating the expression of PLUNC proteins in salivary glands of AIDS patients with or without infectious lesions. The objectives of this study, therefore, were to analyse and compare the expression of PLUNC proteins in salivary glands from patients with and without AIDS using autopsy material collected in the Department of Pathology, São Paulo Medical School from 1996 to 2000.

Materials and methods

Patient population

Ethics Committee approval for the use of autopsy samples was given by Piracicaba Dental School, University of Campinas and São Paulo Medical School, University of São Paulo.

A total of 35 parotid glands, 37 submandibular glands and 27 sublingual glands of patients who had died of AIDS were used in this study. Histological analysis of all cases used in this research has previously been reported (Vargas *et al*, 2003; Rangel *et al*, 2005; Rocha *et al*, 2008; León *et al*, 2009). Clinical records provided information regarding age, gender, and salivary gland related diseases. Patient material was divided into five groups for PLUNC protein expression analysis: group 1 control (HIV negative, 30 cases, 10 cases of parotid gland, 10 cases of submandibular gland and 10 cases of sublingual gland), group 2 HIV+ without histological changes in salivary glands (30 glands, 10 cases of parotid gland, 10 cases of submandibular gland and 10 cases of sublingual), group 3 HIV+ with mycobacteriosis (21 cases, 8 cases of parotid gland, 8 cases of submandibular gland and 5 cases of sublingual gland), group 4 HIV+ with CMV (14 cases, 5 cases of parotid gland, 8 cases of submandibular gland and 1 case of sublingual gland) and group 5 HIV+ with sialadenitis (30 cases, 10 cases of parotid gland, 10 cases of submandibular gland and 10 cases of sublingual gland). In groups 3 and 4, two different regions were analysed: (1) areas around the granuloma or glandular parenchyma where the tuberculosis bacillus or CMV infection was previously detected and (2) areas in the periphery of the lesion at least one field of view (200 ×) distant.

Antibodies

SPLUNC 1 polyclonal rabbit antibody was a generous gift from Dr P Whitney, University of Miami. Two SPLUNC 2 and one LPLUNC 1 affinity purified, peptide-specific, polyclonal antibodies were generated by Eurogentec (Seraing, Belgium) using established methods. The SPLUNC 2 antibodies were labelled A and B and the peptide sequence used for SPLUNC 2A corresponded to amino acids 156–168: (VTIETDPQTHQPV), for SPLUNC 2B corresponded to amino acids 236–249-COOH: (VDNPQHKTQLQTLI) and for LPLUNC 1 corresponded to amino acids, 472–484: (ASLWKPSSPVSQ) at the extreme C-terminus of the protein in the second BPI domain. These peptides were chosen so as to have minimal sequence conservation between man and mouse and no similarity with other members of the PLUNC family.

Immunohistochemistry

All immunohistochemical reactions followed standard protocols. Sections were deparaffinized and rehydrated with xylene and 100% alcohol. Endogenous peroxidase activity was blocked by quenching in 3% H_2O_2 in methanol for 20 min. The sections were rinsed in PBS and where necessary (antibodies SPLUNC 2A and LPLUNC 1) antigen retrieval was performed by microwaving for 8 min in 0.01 M sodium citrate buffer. After a further rinse in PBS sections were incubated in 100% normal serum (corresponding to the species of the secondary antibody) for 30 min at room temperature in a humidified chamber. The serum was drained and replaced with primary antibody diluted in the same 100% normal serum (1:300 SPLUNC 1, 1:250 SPLUNC 2A, 1:500 SPLUNC 2B and 1:600 LPLUNC 1), which was left on the sections overnight at 4°C in a humidified chamber. A Vectastain kit (Vectastain Elite ABC kit, Vector Laboratories, Peterborough, UK) was used for the secondary antibody (according to the manufacturer's instructions) and a VectorRed Kit (Vector NovaRed, Vector Laboratories, Peterborough, UK) was used for colour development. Expression of PLUNC proteins was classified as either positive or negative and positive reactions were further quantified by intensity of cytoplasmic marking (weak, moderate or severe) using Image-Pro Plus version 4.5.

In situ hybridization for SPLUNC 2

RNA extracted from submandibular gland was used as template for amplification by the polymerase chain reaction (PCR) with specific SPLUNC 2 primers (forward primer 5′ AACTGATCCCCAGACACACC 3′ and reverse 5′ GACAATGGGCCTTTATTGC 3′). Following agarose gel electrophoresis DNA was extracted using a QIAquick kit (Qiagen, Crawley, UK), according to the manufacturer's instructions. The DNA was cloned into pCR ^{TM}II vector (Invitrogen,

Carlsbad, CA, USA) following the manufacturer's instructions and following growth in LB broth the plasmid was purified using a QIAprep kit (Qiagen). An ECO-R1 digest (Promega, Southampton, UK) was used to verify correct orientation of the plasmid DNA. Plasmid DNA was linearized with the Hind III and Xho I restriction enzymes and a DIG RNA Labelling Kit (Roche Science, Burgess Hill, UK) was used to synthesize digoxigenin-labelled sense and antisense riboprobes. In situ hybridization was performed as described previously (Lu and Jacobson, 2007) with minor modifications. Paraffin-embedded tissue sections (3 μm) were dewaxed by treatment with xylene (3 x 15 min), hydrated with 99%, 95% and 75% alcohol (5 min each), and washed with water and PBS (10 min each). The sections were then treated with proteinase K (250 μg/ml, 37°C for 35 min, Sigma Aldrich, Dorset, UK), postfixed in 4% paraformaldehyde and 50 μl prehybridization mix (Sigma Aldrich) added for 30 min at 60°C. 50 μl of hybridization solution (prehybridization solution plus digoxigenin-labelled riboprobe) was added to the section and covered with a glass coverslip before incubation at 60°C overnight. The sections were washed with 2xSSC (Sodium Chloride, Sodium Citrate, Sigma Aldrich) at room temperature, twice with 25% formamide in 2xSSC at 45°C for 30 min and then with 2xSSC at 45°C for 30 min. Hybridized probe was detected using the DIG nucleic acid detection kit (Roche Science, UK). Controls run in parallel with each experiment included tissue sections that were incubated in hybridization mix without probe. None of the control sections showed staining.

Statistics

Statistical analysis was performed used parametric and non-parametric methods, Kruskal–Wallis and Mann–Whitney *t*-test and ANOVA for comparison between groups. We used SPSS version 13.0. The test was considered statistically significant when P value is $<$ or equal to 0.05.

Results

Patient population

There were ten males and four females in the control group. None of the patients showed evidence of salivary gland related complaints, gland enlargement or salivary gland disease prior to death.

There were 43 males and 20 females in the AIDS group. The CD4 level prior to death was obtained for 44 patients, with a median of 63.2 cells/μl. None of the patients showed evidence of salivary gland related complaints, gland enlargement or salivary gland disease prior to death.

The average age of patients in the control group was 60.9 years (range 45–70), whilst that of autopsied AIDS patients was 37.8 years (range 15–69) confirming that AIDS is more prevalent in younger adults (World Health Organization, 2009; Rana *et al*, 2000; Eza *et al*, 2003). According to the Ministry of Health in Brazil, in 1985 the ratio of HIV-infected males: females were 25:1, whilst at the end of 2007 this ratio was 2:1. In the present study the male:female ratio was also 2:1.

The patients were divided into five groups for analysis of PLUNC protein expression in salivary glands: group 1 control (HIV negative), group 2 HIV positive (HIV+) without changes in the salivary glands, group 3 HIV+ with mycobacteriosis, group 4 HIV+ with CMV and group 5 HIV+ with sialadenitis.

Immunohistochemistry

SPLUNC 1 – None of the parotid glands in any of the five groups expressed SPLUNC 1. Staining was seen in mucosal acinar cells of the submandibular and sublingual glands but there were no statistically significant differences between the five groups of patients.

SPLUNC 2A – The SPLUNC 2A antibody stained serous acinar cells in parotid, submandibular and sublingual glands. There were no differences in staining in groups 1 and 2 but in the mycobacteriosis group, increased staining was seen around the lesion ($P = 0.019$) compared to the periphery ($P = 0.404$) (Figures 1A, B and 2). The parotid gland appeared to show more intense staining than either the submandibular or sublingual glands but this was not statistically significant (Figure 3). In the CMV group the intensity of staining was statistically significant in ($P = 0.003$) when comparing areas around the lesion with peripheral areas (0.964) (Figure 4A and B). Only 1 case of CMV was positive in the sublingual gland and thus results were compared between submandibular and parotid glands.

SPLUNC 2B – SPLUNC 2B antibody is raised to an epitope in the middle of the protein whilst antibody A is raised to an epitope at the C terminus of the protein. Our previous studies have indicated differential expression patterns with these two antibodies in normal

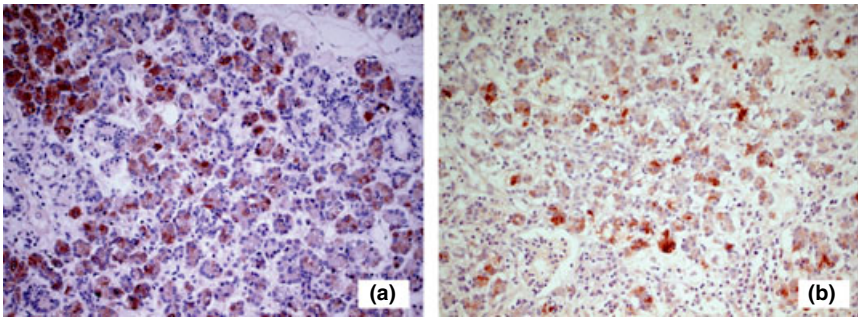

Figure 1 SPLUNC 2A. Parotid gland affected by mycobacteriosis. **(a)** Observe the intense positivity for SPLUNC 2A in the serous cells (brown colour – area considered as around the lesion). **(b)** Moderate positivity for SPLUNC 2A in the serous cells (brown colour – area considered as periphery of the lesion) (streptavidin biotin peroxidase, 200x)

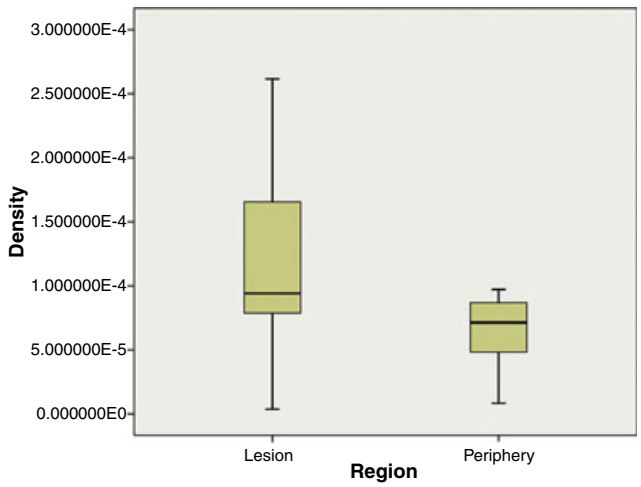

Figure 2 SPLUNC 2A. Difference in staining intensity detected in the area around the lesion and in the peripheral area of Mycobacteriosis cases that affected salivary glands of HIV patients. The intensity was measured as pixel/um

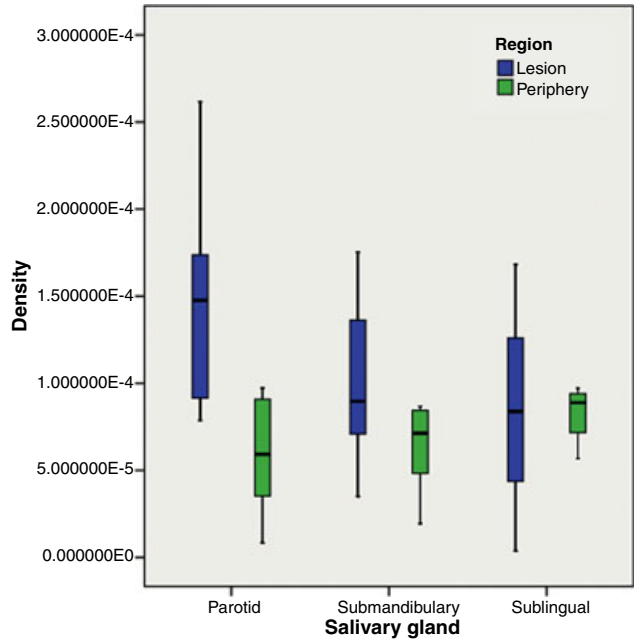

Figure 3 SPLUNC 2A. Comparison of the intensity of staining in the area around the lesion (blue colour) and in the peripheral area (green colour) among major salivary glands infected by *Mycobacterium tuberculosis*

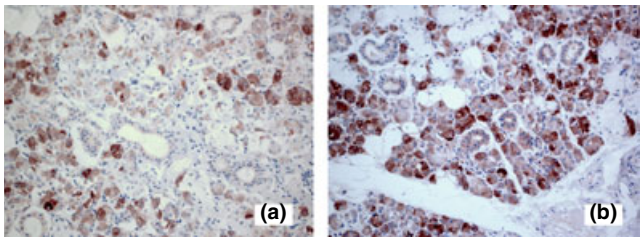

Figure 4 SPLUNC 2A. Submandibular gland affected by CMV. **(a)** Observe the immunoreactivity for SPLUNC 2A in the area around the lesion (brown colour); **(b)** periphery of the lesion (brown colour) (streptavidin biotin peroxidase, 200x)

salivary glands. Thus we were interested to note any differences in expression in the diseased glands used in this study (Vargas *et al*, 2003; León *et al*, 2009). As with antibody A positive staining was seen in serous acinar cells of major salivary glands (Figure 5A–C) and statistically significant differences were again only observed in the mycobacteriosis and CMV groups when comparing intensity between the lesions ($P = 0.0149$) and peripheral areas($P = 0.210$) for mycobacteriosis group and $P = 0.017$ around the lesion and $P = 0.215$ in peripheral areas in CMV infection (Figure 6A and B). Although the stained area was larger in all cases of mycobacteriosis and CMV groups the differences were not significant.

LPLUNC 1 – LPLUNC 1 antibody was positive only in the intercalated, striated and excretory ducts, being completely negative in serous and mucous acinar cells (Figure 7A–C). In the control group 7 of the 10 parotid glands showed positivity and 8 of the10 for submandibular and sublingual glands. In HIV positive patients without alteration immunoreactivity was seen in six parotid glands, seven submandibular and all of the sublingual glands evaluated. Only two parotid glands and three submandibular glands in the Mycobacteriosis group stained positively but again all of the sublingual glands stained for LPLUNC 1. In the CMV group no staining was found in the parotid glands and only three submandibular and one sublingual gland stained positively. The sialedenitis group had four parotid, three submandibular and three sublingual glands which stained positively for LPLUNC 1.

In situ hybridization for SPLUNC 2 in control cases – All cases ($n = 30$) were positive for SPLUNC 2, with positive reactions being seen in the perinuclear region (Figure 8). We observed marked, and more intense, punctate staining in the periphery of serous acinar cells of all cases.

Discussion

Several recent studies have evaluated systemic diseases present in autopsied patients with AIDS (Drut *et al*, 1997; Hsiao *et al*, 1997; Kaiser *et al*, 2000; Ohtomo *et al*, 2000; Eza *et al*, 2006) with a special emphasis on the impact of antiretroviral therapy (Masliah *et al*, 2000); very few studies have evaluated the involvement of the major salivary glands (Ihrler *et al*, 1996; Vargas *et al*, 2003; León *et al*, 2009).

As in previous studies (Bingle *et al*, 2005) we found no SPLUNC 1 in parotid glands and although mucous acinar cells of submandibular and sublingual glands stained positively there were no statistically significant differences in any group in either intensity of staining or area stained.

SPLUNC 2A and 2B antibodies were raised to different epitopes in the protein and we have previously found different staining patterns in normal salivary glands (Bingle *et al*, 2005, 2009). In this study, however, a both antibodies showed intense, positive staining in serous acinar cells in all five groups we compared. Significant differences were only found in cases of

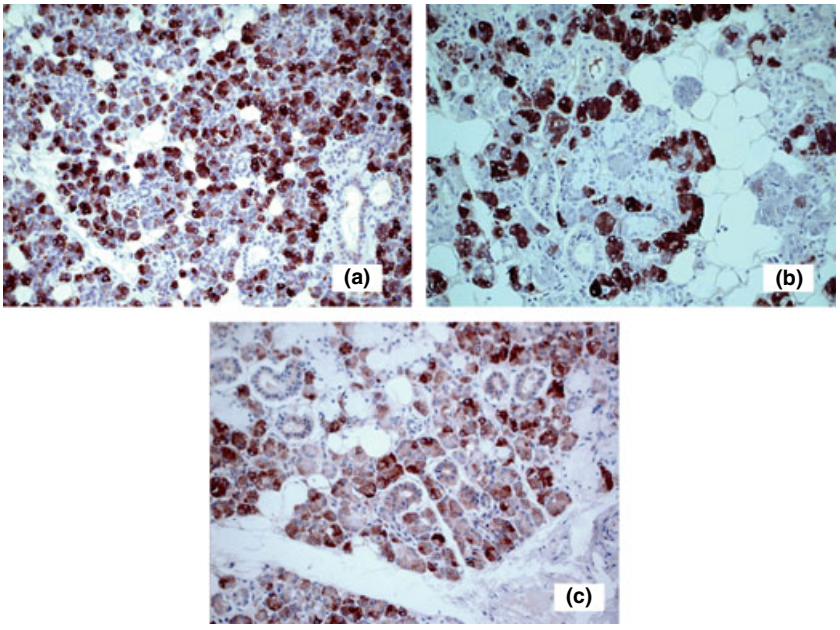

Figure 5 SPLUNC 2B. Intense immunoreactivity for SPLUNC 2B (brown colour) in the serous cells of AIDS patients: **(a)** parotid gland; **(b)** submandibular gland; **(c)** sublingual gland (streptavidin biotin peroxidase, 200x)

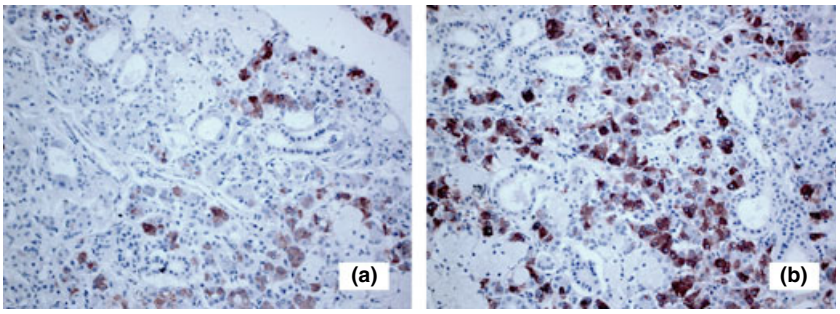

Figure 6 SPLUNC 2B. Submandibular gland affected by CMV. **(a)** Observe the immunoreactivity for SPLUNC 2B in the serous cells (brown colour – area considered as around the lesion). **(b)** Immunoreactivity for SPLUNC 2A in the serous cells (brown colour – area considered as periphery of the lesion) (streptavidin biotin peroxidase, 200x)

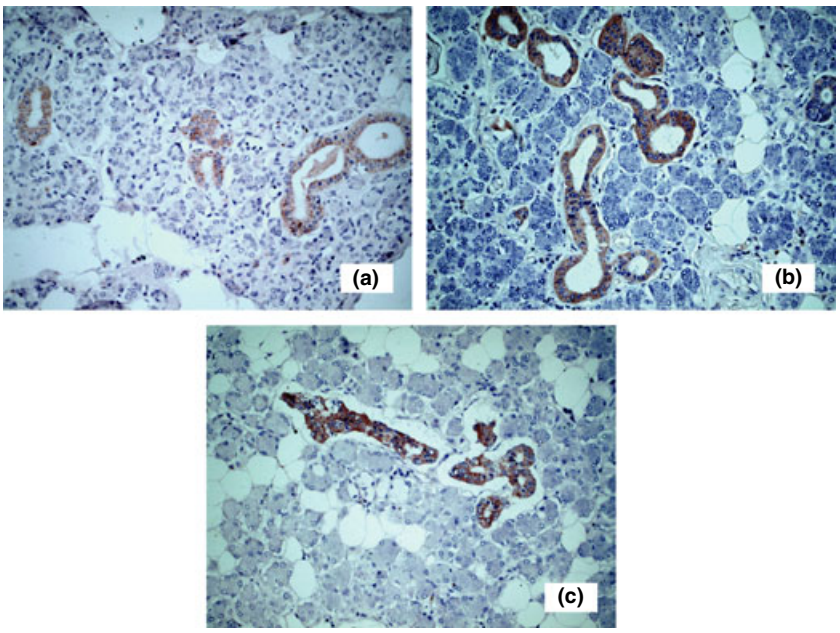

Figure 7 LPLUNC 1 expression in major salivary glands of AIDS patients: **(a)** parotid gland; **(b)** submandibular gland; **(c)** sublingual gland. Note that only duct cells (brown colour) were positive for LPLUNC 1 in the major salivary glands (streptavidin biotin peroxidase, 200x)

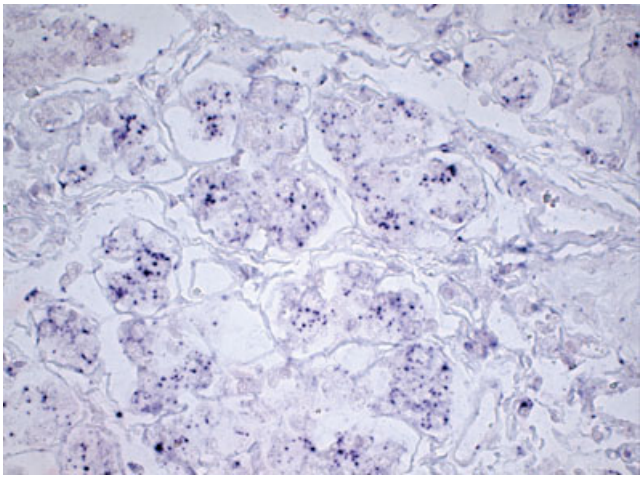

Figure 8 Perinuclear positivity (blue dots) for SPLUNC2 in the serous cells of the submandibular gland in a HIV- negative patient (*in situ* hybridization, 400x)

infection with MTB and CMV, again when comparing with regions around the periphery of the lesion. Vargas *et al* (2008) noted positive SPLUNC 2 staining in 'plugs' of mucin, mucous cells and intermediate cells of mucoepidermoid carcinomas, but in this study we found no staining of mucous cells. This is the first study showing the expression of these proteins in patients with AIDS and thus more research is needed to confirm our findings.

Similarly with LPLUNC 1, Vargas *et al* (2008) found positive staining in 'plugs' of mucin, mucous cells and intermediate cells of mucoepidermoid carcinomas whereas we only saw staining in striated and excretory ducts; no staining in mucosal cells.

This is the first study showing the expression of PLUNC proteins in CMV infections and we found that in CMV-infected tissue the PLUNC expression was more intense around the lesion than within the lesion, although the area near the infection stained was smaller in comparison to the outlying areas. CMV infects mainly ducts, and does not cause a granulomatous reaction, this may account for the larger area stained in the periphery and might suggest a function for PLUNC proteins in protection against infection. According to Bingle and Craven (2002) and LeClair (2003) PLUNC proteins, like other family members (notably BPI and LBP) can potentially bind lipopolysaccharide (LPS). Thus LPLUNC 1 may act in a manner similar to LBP, resulting in a pro-inflammatory response (Bingle 2004) via toll-like receptor signalling, whilst SPLUNC 2 may have an anti-inflammatory function similar to BPI, reducing the microbial load through direct cell death or sequestration of inflammatory molecules.

Tuberculosis is one of the most important opportunistic infections in HIV-infected individuals (Cantwell *et al*, 1994) aided by ineffective therapy in patients with AIDS and interactions with antiretroviral drugs (Jerant *et al*, 2000). Patients with glandular involvement have no specific symptoms of tuberculosis, especially in the absence of a history of pulmonary tuberculosis (Comech-Cerverón *et al*, 1995). The histological pattern reflects the cellular immunity of patients (Lewin-Smith *et al*, 1998) and in our study all patients had low CD4 counts. Among the major salivary glands, the parotid gland is the most commonly affected by Mycobacterium tuberculosis (Mullins *et al*, 2000), whilst others believe that the parotid gland is the most affected (Vargas *et al*, 2003). Our findings show increased expression of SPLUNC 2 in regions near the mycobacterial lesion, similar to the result seen in CMV infection, again suggesting the increased expression in the peripheral regions could be protecting from infection. We propose that, in both CMV and mycobacterial infection, PLUNC proteins protect epithelial cells against infection.

In situ hybridization is a more sensitive technique than immunohistochemistry and no previous studies of PLUNC gene expression in human adult salivary glands has been reported. We performed *in situ* hybridization for SPLUNC 2 only in control tissue and all cases were positive with labelling being seen in the perinuclear region of acinar cells. Further studies are needed to better understand PLUNC protein expression both in normal and diseased tissue.

In conclusion, this is first study of PLUNC protein expression in the salivary glands of autopsied patients with AIDS. PLUNC protein expression was seen in the parotid, submandibular and sublingual glands of patients both with and without AIDS and differential staining was seen in those patients with CMV or mycobacterial infections. Further studies to quantify the amount of PLUNC in saliva and salivary glands would confirm our findings and give a better understanding of the role of these proteins in the control of CMV and mycobacterial infections.

Acknowledgements

This work was supported by The State of São Paulo Research Foundation (FAPESP) and The National Council for Scientific and Technological Development (CNPq).

References

Bingle CD, Bingle L (2000). Characterization of the human PLUNC gene, a gene product with an upper airwarys and nasopharygeal restricted expression pattern. *Biochim Biophys Acta* **1493:** 363–367.

Bingle CD, Craven J (2002). PLUNC: a novel family of candidate host defense proteins expressed in the upper airways and nasopharynx. *Hum Mol Genet* **11:** 932–943.

Bingle CD, LeClair EE, Havard S, Bingle L, Gillingham P, Craven CJ (2004). Phylogenetic and evolutionary analysis of the PLUNC gene family. *Protein Sci* **13:** 422–430.

Bingle L, Cross SS, High AS *et al* (2005). SPLUNC1 (PLUNC) is expressed in glandular tissues of the respiratory tract and in lung tumours with a glandular phenotype. *J Pathol* **205:** 491–497.

Bingle L, Barnes FA, Lunn H *et al* (2009). Characterisation and expression of SPLUNC2, the human orthologue of rodent parotid secretory protein. *Histochem Cell Biol* **132:** 339–349.

Cantwell MF, Snider DE Jr, Cauthen GM, Onorato IM (1994).Epidemiology of tuberculosis in the United States, 1985 through 1992. *JAMA* **272:** 535–539.

Comech-Cerverón C, Barona de Guzmán R, Martinez LE (1995). Parotid tuberculosis. *Acta Otorrinolaringol Esp* **46:** 387–390.

De Faria PR, Vargas PA, Saldiva PHN, Bohm GM, Mauad T, de Almeida OP (2005). Tongue disease in advanced AIDS. *Oral Dis* **11:** 72–80.

Drut R, Anderson V, Greco MA *et al* (1997). Oportunistic infections in pediatric HIV infection: a study of 74 autopsy cases from Latin America. The Latin America AIDS Pathology Study Group. *Pediatr Pathol Lab Med* **17:** 569–576.

Elvira J, Garcia del Rio E, Lopez-Suarez A, Garcia-Matos P, Giron JA (1998). Submandibular gland infection by *Mycobacterium avium-intracellulare* in an AIDS patient. *Eur J Clin Microbiol Infect Dis* **17:** 529–535.

Eza D, Cerrillo G, Moore DAJ *et al* (2006). Postmortem findinggs and opportunistic infections in HIV-positive patients from a public hospital in Peru. *Pathol Res Pract* **202:** 767–775.

Gomes RT, Naves MD, Carmo MAV, Aguiar MCF (2006). Sialoadenites: revisão de literatura sobre a etiologia, o diagnóstico e o tratamento. *Arq Odont* **42:** 288–296.

Greenspan D, Komaroff E, Redford M *et al* (2000). Oral mucosal lesions and HIV viral load in the Women's Interagency HIV Study (WIHS). *J Acquir Immune Defic Syndr* **25:** 44–50.

Hernández SL, López de Blanc AS, Sambuelli RH *et al* (2004). Oral histoplasmosis associated with HIV infection: a comparative study. *J Oral Pathol Med* **33:** 445–450.

Hsiao CH, Huang SH, Huang SF *et al* (1997). Autopsy finding on patients with AIDS in Taiwan. *J Microbiol Immunol Infect* **30:** 145–159.

Ihrler S, Zietz C, Riederer A, Diebold J, Lohrs U (1996). HIV-related parotid lymphoephitelial cysts. Immunohistochemistry and 3-D reconstruction of surgical and autopsy material with special reference to formal pathogenesis. *Virchows Arch* **429:** 139–147.

Jerant AF, Bannon M, Rittenhouse S (2000). Identification and management of tuberculosis. *Am Fam Phys* **61:** 2667–2678.

Joshi SM, Pandey AK, Capite N, Fortune SM, Rubin EJ, Sassetti CM (2006). Characterization of mycobacterial virulence genes through genetic interaction mapping. *Proc Natl Acad Sci USA* **103:** 11760–11765.

Kaiser A, Weng LP, Brockhaus W, Wunsch PH (2000). Oportunistic infections and HIV-associated malignancies. An evaluation of 58 autopsy case within 10 years. *Med Klin* **95:** 482–486.

LeClair EE (2003). Four BPI (bactericidal/permeability-increasing protein)-like genes expressed in the mouse nasal, oral, airway and digestive epithelia. *Biochem Soc Trans* **31:** 801–805.

León JE, Mauad T, Saldiva PH, Almeida OP, Vargas PA (2009). Submandibular and sublingual glands involvement in advanced acquired immunodeficiency syndrome (AIDS): an autopsy-based study. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* **108:** 216–226.

Lewin-Smith MR, Klassem MK, Frankel SS, Nelson AM (1998). Pathology of human immunodeficiency virus infection: infection conditions. *Ann Diagn Pathol* **2:** 181–194.

Lu FX, Jacobson RS (2007). Oral mucosal immunity and HIV/SIV infection. *J Dent Res* **86:** 216–226.

Masliah E, DeTeresa RM, Mallory ME, Hansen LA (2000). Changes in pathological findings at autopsy in AIDS cases for the last 15 years. *AIDS* **14:** 69–74.

Miziara ID, Araujo Filho BC, Weber R (2005). AIDS and recurrent aphthous stomatitis. *Braz J Otorhinolaryngol* **71:** 517–520.

Monteil RA, Holfman P, Michiels JF, Labouyrie R (1997) Oral cryptococcosis: case report of salivary gland involvement in an AIDS patient. *J Oral Pathol Med* **26:** 53–56.

Mullins JE, Ogle O, Cottrel DA (2000). Painless mass in the parotid region. *J Oral Maxillofac Surg* **58:** 316–319.

Ohtomo K, Wang S, Masunaga A, Aikichi I, Sugawara I (2000). Secondary infections of AIDS autopsy cases in Japan with special emphasis on *Mycobacterium avium–intracellulare complex* infection. *Tohoku J Exp Med* **192:** 99–109.

Raab SS, Thomas PA, Cohen MB (1994). Fine-needle aspiration biopsy of salivary gland mycoses. *Diagn Cytopathol* **11:** 286–290.

Rana FS, Hawken MP, Mwachari C *et al* (2000). Autopsy study of HIV-1-positive and HIV-1-negative adult medical patients in Nairobi, Kenya. *J Acquir Immune Defic Syndr* **24:** 23–29.

Rangel AL, Coletta RD, Almeida OP *et al* (2005). Parotid mycobacteriosis is frequently caused by *Mycobacterium tuberculosis* in advanced AIDS. *J Oral Pathol Med* **34:** 407–412.

Rocha LA, Vargas PA, Silva LF *et al* (2008). Expression of secretory leukocyte proteinase inhibitor in the submandibular glands of AIDS patients. *Oral Dis* **14:** 82–88.

Singh B, Balwally NA, Har-El G, Lucente FE (1998). Isolated cervical tuberculosis in patients with HIV infection. *Otolarygol Head Neck Surg* **118:** 766–770.

Vargas PA, Mauad T, Bohm GM, Saldiva PH, Almeida OP (2003). Parotid gland involvement in advanced AIDS. *Oral Dis* **9:** 55–61.

Vargas PA, Speight PM, Bingle CD, Barret AW, Bingle L (2008). Expression of PLUNC family members in benign and malignant salivary gland tumours. *Oral Dis* **14:** 613–619.

Wagner RP, Tian H, McPherson MJ, Latham PS, Orenstein JM (1996). AIDS-associated infections in salivary gland: autopsy survey of 60 cases. *Clin Infect Dis* **22:** 369–371.

Weston WM, LeClair EE, Trzyna W *et al* (1999). Differential display identification of plunc, a novel gene expressed in embryonic palate, nasal epithelium and adult lung. *J Biol Chem* **274:** 13698–13703.

World Health Organization (2009) *UNAIDS AIDS epidemic update*. Joint United Nations Programmen on HIV/AIDS, Geneva, Switzerland. November 1–3.

Yuan HT, Gowan S, Kelly FJ, Bingle CD (1997). Cloning of guinea pig surfactant protein A defines a distinct cellular distribution pattern within the lung. *Am J Physiol* **273:** 900–906.