## 3129

Origin of Strains Causing Oral Thrush in HIV-Positive Individuals. K. VARGAS*, S. LOCKHART, J. HELLSTEIN, M. NICHOLS and D.R. SOLL (The University of Iowa, Iowa City, IA)

One of the first opportunistic infections in HIV-associated disease is oral-esophageal candidiasis. It has been assumed that the initial infection involves the conversion of the host commensal to a pathogen. However, there has been no genetic analysis to support this conclusion. We have, therefore, analyzed the genetic relatedness of oral yeast isolates from 10 HIV-positive individuals collected before the first episode of thrush, at the time of the first episode and at various times afterwards. Isolates were fingerprinted by Southern blot hybridization with the moderately repetitive DNA fingerprinting probe Ca3. Patterns were analyzed with the computer-assisted DNA fingerprint analysis system Dendron. Similarity coefficients ($S_{AB}$) based on band intensity and position were computed for every pair, and dendrograms were generated based upon these values. The results showed that strain maintenance occurred in 60% of the cases. However, in 40%, replacement of the commensal strain with a different strain occurred. It can be concluded, therefore, that strain adaptation of the yeast to changes in the environment caused by progressive immunodeficiency may be occurring in the oral cavities of HIV-positive individuals that exhibited strain replacement. A similar change may also be occuring in the persons exhibiting strain maintenance. However, since this involves phenotypic variation as opposed to genotypic, it can not be measured by fingerprinting. This research was supported by NIH grant DE00364.

## 3130

Relative Cell Surface Hydrophobicity of *Candida parapsilosis* and *Candida albicans*. G.J. PANAGODA* & L.P. SAMARANAYAKE (Oral Biology Unit, Faculty of Dentistry, The University of Hong Kong, Hong Kong).

The cell surface hydrophobicity (CSH) is regarded as an important physical force governing adhesion of microbes to various biological and non-biological surfaces. *C. parapsilosis* is an emerging pathogen and together with *C. albicans* cause diseases such as oral thrush, fungemia and endocarditis. As the CSH of these yeasts appear to be important in the pathogenesis of candidoses, 24 (15 superficial - oral/cutaneous and 9 systemic - blood) isolates of *C. parapsilosis* and 5 oral *C. albicans* isolates were studied using a biphasic (hydrocarbon/aqueous) separation assay (Samaranayake *et al.*, APMIS, 1995, 103: 707 - 713). A yeast suspension of 5 ml ($10^7$ yeasts/ ml) with an absorbance of 1.0 ± 0.02 was mixed with 1 ml of xylene and incubated for 10 min at 37° C. A suspension free of xylene acted as the control. After vortex-mixing for 30 s and further incubating for 30 min to separate the aqueous and the hydrocarbon phase, the absorbance values of the aqueous phase of the test and control tubes were measured. The CSH was expressed as the percentage drop in optical density of the test suspension compared with the control. The CSH of *C. parapsilosis* (56.4%), was significantly greater than that of *C. albicans* (20%; $p < 0.0001$). The superficial isolates of *C. parapsilosis* demonstrated very high CSH (64.4%) compared with the systemic isolates (43.1%, $p < 0.0001$). When CSH of oral (66.6%) and cutaneous (62.9%) isolates of *C. parapsilosis* was compared, no significant difference was noted. This study suggests the existence of interspecies differences in CSH in *Candida* species and, even within a given species the habitat in which they reside may modulate this parameter. Supported by Research Grants Council of Hong Kong.

## 3131

Surveillance of Antifungal MIC Values of Oral Yeast From AIDS Subjects. PAULETTE J. TEMPRO*, ANDRE BARASCH, LYNN MIKULSKI (University at Buffalo School of Dental Medicine and University of Medicine and Dentistry of New Jersey)

Antifungal resistance may emerge in oropharyngeal yeast in immunocompromised host on long-term antifungal therapy. This study was conducted to evaluate colonization with yeast of oral sites in AIDS subjects on maintenance antifungal agents and level of susceptibility to amphotericin B (AP), flucytosine (FL), ketoconazole (KE), fluconazole (FL) and itraconazole (IT) of the yeast isolates. Mucosal samples were obtained from 4 adults and 6 children with AIDS and clinical evidence of oropharyngeal candida infection. The medical regimen included antibacterial, antifungal and antiviral chemotherapeutic treatment. Samples were plated on CHROMagar® and genus and species of isolates were determined by performing germ tube test, observing cornmeal agar morphology, carbohydrate assimilation pattern and urease production. Candida was isolated from all 10 subjects, 5 subjects harbored only Candida albicans and 5 had mixed colonization consisting of C. albicans, Candida (Torulopsis) glabrata and Candida parapsilosis. The minimal inhibitory concentration (MIC) values for 17 representative isolates were determined using AP, 5FC, KE, FL and IT ETest® strips. C. albicans ETest® MIC values for AP, 5FC, KE, FL and IT were between 0.16-1.5; 0.01-2; 0.004-1.5; 0.75-256 and 0.064-32 ug/ml respectively. C. glabrata and C. parapsilosis MIC values for AP and 5FC were uniformly low (between 0.032-0.75ug/ml) and for KE, FL and IT between 0.002-1.0; 0.002->64 and 0.002-16 ug/ml respectively. FL resistant C. albicans (256ug/ml) and C. glabrata (>64 ug/ml) were isolated from two subjects. Both isolates had low MIC values for KE and IT. Related Candida spp. from these two subjects had low FL and azole MIC values. The majority of Candida isolates from AIDS subjects on long-term antifungal azole therapy in this survey had MIC values in the sensitive to moderate range for KE, FL and IT (≤64ug/ml). FL resistance didn't appear to confer resistance to other azoles nor were related spp. resistant in the same subject. Theses results indicate a unique resistance mechanisms for each azole agent and Candida spp. Supported by #DE10156B.

## 3132

Effect of Oral Bacteria on Germ-tube Formation in *C. albicans*. R.G. NAIR* and L.P. SAMARANAYAKE (Oral Biology Unit, Faculty of Dentistry, The University of Hong Kong, Hong Kong)

Germ-tube (GT) formation in *C. albicans* is thought to be an important virulence attribute of this opportunistic oral fungal pathogen. As it is feasible that oral commensal bacteria may play a role in modulating the GT formation in *Candida*, a total of 8 oral bacterial isolates belonging to six species, and a select group of 12 oral *C. albicans* isolates (6 from HIV-infected and 6 from healthy individuals) were used to study the effect of bacteria on GT formation. Briefly, 0.5 ml of bacterial suspension ($10^{5-6}$ cells/ml) was added to equal volume of *C. albicans* suspension ($10^7$ cells/ml) and incubated at 37°C for 90 min. with 0.5 ml of bovine serum. Then the percentage of GT positive *Candida* cells were quantified using a haemocytometer, under X400 magnification. In general, out of eight bacteria, *S. sanguis SK 21A, S. salivarius SK56, E. coli ATCC25922, P. gingivalis Pg50* and *Prevotella intermedia OBU4* suppressed GT formation in varying degrees, in different *C. albicans* isolates. However *S. sanguis OBU2* and *S. salivarius OBU3*, both isolated from HIV-infected individuals elicited significant enhancement and no effect on GT formation, respectively. The current results tend to suggest that commensal oral bacterial populations may selectively influence the differential expression of GT forming ability of *C. albicans* isolates.

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## 3133

Recruitment of Immune Cells in Human Palatal Mucosa in Response to *Candida albicans*. Rowland, RW[1]*, Mackenzie IC[2], Soehren SE[1]. [1]University of Detroit Mercy, Detroit, [2]University of Michigan, Ann Arbor, MI

Candidiasis is the most common oral manifestation of HIV infection. However, the underlying cell mediated immune response to oral candidiasis is poorly understood. The purpose of this study was to evaluate *in situ*, the recruitment of the cell mediated immune system in response to *Candida albicans*. *Candida albicans* strain 209 (CA) was grown overnight in Sabouraud's both, harvested, re-suspended in buffered formalin saline and incubated overnight/4°C. CA were then washed 3x PBS and re-suspended in PBS at $10^{10}$/ml. Informed consent with IRB approval was obtained and a customized palatal stent with 2 equal reservoirs, one for PBS, one for CA preparation, was then placed for 48h in a healthy 42 year old male. 5mm punch biopsies of each area were obtained under local anesthesia and frozen. Frozen sections were cut at 5µ, formalin fixed, and incubated with monoclonal antibodies for HLA-Dr (macrophages, Langerhans cells), CD1a (Langerhans cells), and CD3 (mature T cells), at 1:20 and 1:80 dilutions overnight/4°C, and washed 3x. Sections were then incubated 40min/RT with secondary antibody (IgG coupled to Fluorescein Isothiocyanate), diluted to 1:80, washed, and mounted with anti-fade medium, then viewed at 10x and 20x. Qualitative evaluation found the test site (CA) to exhibit many more cells staining for HLA-Dr, CD1a, and CD3 when compared to the control site (PBS). These preliminary data demonstrate an accumulation of cell types with the appropriate phenotypic characterization in response to mucosal exposure of a *Candida albicans* preparation. Further work will include markers of immune function/activation and quantitative evaluation. Supported by the University of Detroit Mercy, Faculty Research Fund.

## 3134

**Oral Microflora in patients with Sjögren's syndrome.** A. ALMSTÅHL[1]*, U. KRONELD[2], A. TARKOWSKI[2] and M. WIKSTRÖM[1] ([1]Dep of Oral Microbiology, [2]Dep of Rheumatology, University of Göteborg, Sweden)

It is generally assumed that a decreased salivary secretion rate will promote plaque accumulation and increase the risk for gingival inflammation, caries and mucosal infections. In a previous study we found no statistically significant differences in the oral flora between subjects with xerostomia, but with no known underlying disease, and their controls. Patients with Sjögren's syndrome, which is a chronical, inflammatory, auto-immune disease, often have a low secretion due to inflammation in the salivary glands. They have also been reported to have an increased frequency of caries, early dental loss and opportunistic microorganisms. In this study the oral flora in rinsing samples in 10 subjects with primary and 10 subjects with secondary Sjögren's syndrome was compared with the oral flora in controls, matched according to age, sex and number of teeth. In the group with primary Sjögren's syndrome mean salivary secretion rate at rest was 0.008±0.01 ml/min and when stimulated 0.27±0.25 ml/min. Corresponding values in the group with secondary Sjögren's syndrome were 0.03±0.03 and 0.83±0.51 ml/min respectively. Both groups with Sjögren's syndrome showed a tendency, although not statistically significant, to a higher total number of microorganisms and an increased proportion of mutans streptococci. Mean numbers of lactobacilli and *C. albicans* were higher in the group with primary Sjögren's syndrome than in the control group ($p<0.05$). The proportion of lactobacilli, of the total bacterial count, was higher in the group with secondary Sjögren's syndrome than in their control group ($p<0.05$). The mean number of *F. nucleatum* and *P. intermedia* and the frequency of *Staphylococcus aureus* and enterics was similar in all 4 groups. The results support earlier findings that low secretion rate favour aciduric microorgnisms in the oral cavity but do also suggest that an underlying disease may influence on the flora established.

## 3135

Search for *Mycobacterium paratuberculosis* in OFG and oral Crohn's disease. M. P. RIGGIO*, J. GIBSON, A. LENNON, D. WRAY and D. G. MACDONALD (Glasgow Dental School, Scotland, UK).

Although Crohn's disease of the gut has long been suspected to have a mycobacterial cause, a possible mycobacterial involvement in orofacial granulomatosis (OFG) and oral manifestations of Crohn's disease has not yet been investigated. Since the slow-growing *Mycobacterium paratuberculosis* has been implicated in the etiology of gut Crohn's disease, the potential presence of this organism in OFG and oral Crohn's disease tissue was investigated. Polymerase chain reaction (PCR) using primers directed against the 5' region of the IS900 DNA insertion element sequence of *M. paratuberculosis* was used to attempt to detect the organism in tissue samples. The PCR assay was used on archival formalin-fixed paraffin-embedded tissue sections held at Glasgow Dental School. Of the biopsies analyzed, 30 were from patients with OFG, 7 from patients with oral Crohn's disease and 12 from normal control patients. In order to achieve maximum sensitivity, two rounds of PCR were carried out and products were confirmed by hybridization to a digoxigenin-labelled IS900 DNA probe. A single OFG sample was positive for *M. paratuberculosis* and all oral Crohn's disease samples and normal controls were negative. These results suggest that *M. paratuberculosis* is not a major etiological agent in OFG and oral Crohn's disease. Additional studies are being carried out to investigate the prevalence of this organism in OFG and oral Crohn's disease tissue samples from patient groups in several geographical locations in the UK. This study was supported by the Crohn's in Childhood Research Association and the Scottish Higher Education Funding Council.

## 3136

Increase of TNF-α In Cats During Orthodontic Tooth Movement. A. WICHELHAUS*, I. VITOULADITIS, Y. OKAMOTO, H. GOGEN, J. SHANFELD, Z. DAVIDOVITCH (Ulm Univ., Ohio State Univ., Harvard Univ.).

Orthodontic forces generate cytokines that affect the formation and resorption of bone. Tumor necrosis factor-α (TNF-α) is of particular importance as a modulator of bone resorption. The purpose of this study was to determine the possible role of TNF-α in the remodelling of paradental tissues during orthodontic treatment. Three groups of 1-year old male cats had one maxillary canine translated distally by 80 g for 12 h, 7 on 56 d (3 cats/group). Frozen jaws were sectioned sagitally at 6 µm and stained for TNF-α using rabbit polyclonal antibodies. Staining intensity of 10 periodontal ligament (PDL) fibroblasts and 10 alveolar bone surface cells (ABC) in sites of tension and compression, and in corresponding control sites near untreated canines were measured by a computerised photomicroscope. Data was analysed by t-test. Overall the TNF-α staining intensity of control cells was 65.51 ± 3.31 %, while that of cells near treated canines was 82.13 ± 4.88 % ($p < 0.0001$). There was no significant difference between PDL fibroblasts and ABC. In the treated groups, the staining intensity of TNF-α of PDL-fibroblasts and ABC was significantly higher in sites of compression ($p < 0.0001$). The greatest increase in cellular staining occurred at 12 h in both sites of compression and tension. These results suggest that TNF-α plays a role in modulating the response of paradental cells to applied mechanical forces in vivo, and that it is involved in the activity of cell in sites of both resorption and formation. In the latter site, TNF-α may play an inhibitory role.

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