121 TCR Vβ Gene Expression in Oral Lichen Planus Lesional T Lymphocytes. N.W. SAVAGE*, X. ZHOU, P.B. SUGERMAN and L.J. WALSH (Oral Biology and Pathology, Dept. of Dentistry, The University of Queensland, Brisbane, Australia).

Oral lichen planus (OLP) is a T cell-mediated oral mucosal disease. The aim of this study was to test the hypothesis that OLP lesional T lymphocytes demonstrate restricted usage of genes encoding the T cell receptor beta chain variable region (TCR Vβ). T lymphocytes were isolated from OLP lesions as described previously by Sugerman *et al.* (*J Oral Pathol Med* 22:126-31,1993). Total RNA was extracted using acid guanidinium-phenol-chloroform. Following reverse transcription, polymerase chain reaction (PCR)-based analysis of TCR Vβ multigene family was undertaken using a panel of 26 TCR Vβ specific primers. TCR Vβ1, Vβ2, Vβ3, Vβ4, Vβ5.1, Vβ6.1-3, Vβ7, Vβ8, Vβ13.1, Vβ13.2, Vβ14, Vβ15, Vβ17, Vβ19 and Vβ20 were expressed in many cell lines. Vβ5.2-3, Vβ9, Vβ10, Vβ11, Vβ12, Vβ16, Vβ18, Vβ21, Vβ22, Vβ23 and Vβ24 were rarely utilized. The finding of restricted TCR Vβ gene usage suggests oligoclonality within the T lymphocyte population in OLP and that OLP may be an antigen-specific disease. Furthermore, shared TCR Vβ gene usage between patients suggests the involvement of a common antigen in the pathogenesis of OLP. This study was supported by The National Health and Medical Research Council (Australia) and The Australian Dental Research Fund, Incorporated.

122 Gingival Status: A Confounding Variable When Monitoring Lymphocyte Phenotype & Function During HIV Disease. RW ROWLAND*[1], AM COCHRAN[1] and RB FRIEDMAN[2]. [1]U Detroit Mercy, Detroit MI, [2]VCU, Richmond VA, USA

Both gingivitis and periodontitis are associated with changes in the phenotypic profile and function of lymphocytes. Previous studies from this laboratory have shown alterations in cytokine production and lymphocyte function are associated with gingival status and race in non-HIV infected (HIV-) subjects (Rowland, J Periodont Res, 1993) and that necrotizing ulcerative gingivitis (NUG) is associated with HIV infection (Rowland, *et al.*, Clin Infec Dis, 1993). The purpose of this study was to investigate the effects of gingival status and HIV disease on CD4 and CD8 counts, mitogenic response, and serum levels of sIL2R, IL2, and IL4. HIV infected (HIV+) subjects diagnosed with gingival health or slight gingivitis (H/G) and NUG were compared to HIV- control subjects of similar periodontal clinical status: HIV-NUG (n=17), HIV+NUG (n=4), HIV+H/G (n=12), HIV-H/G (n=22). Nineteen non-HIV infected individuals with severe gingivitis (SG) were also included as a control group. Phenotypes were determined by flow cytometry, mitogenic response by incorporation of ^{3}H-thymidine in response to phytohemagglutinin stimulation, cytokines & receptors by bioassays and ELISA. Significant differences ($p \leq 0.05$) in the parameters measured were found between some of the clinical groups: CD4 & CD8: HIV-NUG, HIV-H/G, HIV+NUG, and HIV+H/G; Mitogenic Response: HIV-SG, HIV-NUG, and HIV+NUG; sIL2R: HIV+H/G, HIV-NUG, and HIV-SG; IL4: HIV+H/G, HIV-H/G, HIV-NUG, and HIV-SG. From these data, clinical gingival status appears to be a confounding variable when measuring lymphocyte phenotypes and function in the presence of HIV disease.
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123 Parameters affecting the adhesion of *Candida albicans* to buccal cells in HIV infection L.P. SAMARANAYAKE*, P.C.S. TSANG, S.S. LEE AND P. LI (University of Hong Kong, Queen Elizabeth Hospital and Department of Health, Hong Kong).

Although oral *Candida* infections herald the onset of HIV disease, there is little data on the pathogenesis of oral candidosis in this infection. Therefore, the adhesion of *C. albicans* to buccal epithelial cells (BEC) of 23 HIV-infected individuals and 26 healthy controls was compared as mucosal adhesion is an essential prerequisite for colonisation leading to oral candidosis. BEC, collected from the cheek mucosa of these individuals were suspended in phosphate buffered saline and incubated with a suspension of *C. albicans* GDH1957 (10^7yeasts/ml) for 37°C, for 30 min. Subsequently the BEC were washed once with buffer, Gram stained, and the number of yeasts attached to 100 BEC were quantified by microscopy (Infect Immun 1979;21:24-8). In addition to comparing the candidal adhesion to healthy and control groups, the effect of other parameters including the age group, CDC classification, CD4 level, antiviral therapy and antifungal therapy on candidal adhesion was also evaluated. The results indicated a three-fold increased avidity of *Candida* to BEC of infected individuals ($p < 0.001$), and a two-fold increased adhesion of *Candida* to BEC of individuals on antiviral therapy (didanosine) as opposed to those who were not ($p < 0.05$). None of the other parameters investigated, ie. age, CDC classification, CD4 level and antifungal therapy, significantly influenced candidal adhesion to BEC. These data indicate that BEC of HIV-infected individuals may be more receptive to candidal colonisation, and didanosine therapy may, in addition to its antiviral effect, be beneficial in suppressing candidal colonisation of the oral mucosa in HIV infection.
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124 Detection of *Mycoplasma genitalium* in saliva by PCR. S. McCORMACK, M. I. CHINGBINGYONG and C.V. HUGHES* (Pediatric Dentistry, Boston University, Boston, MA USA)

Mycoplasma genitalium was initially identified from the urogenital tract of men with nongonococcal urethritis. Subsequently isolated in samples from Pelvic Inflammatory Disease as well as pneumonias, its tissue tropism remains a major question. Recently, a method of detection of *M. genitalium* (Skov-Jensen et al, J Clin Micro., 1991) has been developed that uses the polymerase chain reaction method to amplify a 281-basepair region unique to *M. genitalium* from the structural gene of a 140-kDa adhesin. In the present study, we used this method to detect *M. genitalium* in whole saliva and test the hypothesis that the mouth may serve as a reservoir for the organism in human populations. DNA was purified from saliva samples obtained from 56 healthy adults and tested for the presence of *M. genitalium* by PCR. Each test sample was examined for the expected 281-basepair amplification product by ethidium bromide staining of 6% polyacrylamide gels. Results were confirmed by Southern hybridization with a 148-basepair digoxigenin-labeled probe based on an internal sequence of the expected amplification product. The 281-basepair amplification product was detected in 45 of 56 (80.4%) samples tested. These data suggest that *M. genitalium* is present in the saliva of many human subjects and that the mouth may serve as a reservoir for the organism. It also suggests that saliva may play a role in its transmission between individuals.

125 Expression of Matrix Components During Cementoblast Differentiation. J.-W. SHIN, W.-L. LIN, M.-I. CHO* (Department of Oral Biology, Periodontal Disease Research Center, State University of New York at Buffalo, New York, USA).

Previously, we reported sequential differentiation of the dental follicle proper mesenchymal cells into precementoblasts and cementoblasts during formation of acellular extrinsic fiber cementum (AEFC) in the rat (Anat. Rec. 223:209, 1989). Using immunohistochemistry (Ih) and immunogold labeling (IL), we now study the expression of extracellular matrix (ECM) components during cementoblast differentiation. After perfusion of two-wk old rats with 3% paraformaldehyde-0.1% glutaraldehyde, the first maxillary molars and periodontal tissues were removed, dehydrated, and embedded in paraffin for Ih or in Lowicryl K4M resin for IL. To localize ECM components, 6-μm thick paraffin and ultrathin Lowicryl sections were incubated with antibodies to type I collagen (CI), fibronectin (Fn), SPARC, osteopontin (OPN) or bone sialoprotein (BSP). The results of Ih demonstrated that immunostaining for CI, Fn and SPARC was observed over both unmineralized and mineralized cementum formed by precementoblasts and cementoblasts, respectively. Fn staining was also found on the basement membrane of Hertwig's root sheath epithelial cells. However, staining for OPN and BSP was not observed on unmineralized cementum, but first found on mineralized cementum where cementoblasts are located. Co-localization of OPN and BSP using a double labeling technique demonstrated their localization on electron-dense amorphous matrix components. These results suggest that precementoblasts and cementoblasts synthesize and deposit CI, Fn and SPARC in unmineralized cementum, while cementoblasts synthesize additional ECM components such as OPN and BSP that may play an important role in the initiation or regulation of mineralization of the cementoid. Supported by USPHS Grant DE 04898.

126 Differential Distribution of Lumican and Fibromodulin in Tooth Cementum. H. CHENG, P. NEAME, G. LESTER, B. CATERSON and M. YAMAUCHI* (Dental Research Center, U. North Carolina, Chapel Hill, NC, USA)

The objectives of this study were to isolate and characterize the major proteoglycans (PGs) of cementum that may play roles in this tissue's mineralization. Cementum was collected from the root apex region of bovine molars and pulverized. It was first extracted with 6M guanidine-HCl, pH 7.4 (G-ext, mineral-unassociated) and then demineralized and extracted with 0.5M EDTA (ED-ext, mineral-associated). Both extracts were applied to anion exchange chromatography and the fractions collected were assayed for chondroitin- (CS) and keratan sulfate (KS) containing PGs using the monocolonal antibodies 2-B-6 and 5-D-4, respectively. It was found that the KS was the major GAG and was enriched in the G-ext fraction. The major KS fraction was further purified and applied to SDS-PAGE. The major broad band (60kD) was 5-D-4 positive in Western blot analysis and separated into two bands (46 and 50 kD) after the treatment with keratanase II and endo-β-galactosidase. These two proteins were transfered to PVDF membrane and analyzed for amino acid sequence. The results indicated the major band (46kD) to be lumican and the minor (50kD) fibromodulin. In addition, based on the immunohistochemical study using 5-D-4, the KS-PGs were found to be located almost exclusively in nonmineralized portion of cementum such as precementum and the pericementocyte area. These data suggest that the major KSPGs of cementum, lumican and fibromodulin, may have inhibitory effects on cementum mineralization.
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127 Alkaline Phosphatase Activity in Rat Periodontium is Related to Cementum Formation. M.C. GROENEVELD, V. EVERTS and W. BEERTSEN*. (Dept. Periodontology, Academic Center for Dentistry Amsterdam (ACTA), The Netherlands).

The enzyme alkaline phosphatase (ALP) is thought to be involved in processes leading to mineralization of hard tissues like bone and cementum. In a previous study of the rat incisor it appeared that highest activity in the periodontal ligament was found adjacent to the alveolar wall and at the site where acellular cementum formation begins, just occlusal to Hertwig's epithelial root sheath (Groeneveld *et al.*, J Dent Res 72:1344-1350, 1993). In an attempt to further establish the relationship between ALP and cementum formation, the activity of the enzyme was quantitatively assessed in the periodontium of rat maxillary molars and related to cementum thickness. The indoxyl-tetrazolium salt method was used to demonstrate enzyme activity in undecalcified sections of 6 μm thickness, cut parallel to the longitudinal axis of the molars. The thickness of the cementum layer was measured by using a calibrated measuring-ocular. The distribution of ALP-activity in the molar periodontium proved to be heterogeneous indicating local variations in phosphate household. Highest activity was found adjacent to the alveolar bone and to the cementum. Enzyme activity was higher adjacent to cellular than to acellular cementum. With respect to acellular cementum, a highly significant positive correlation was found between ALP-activity and cementum thickness ($r = 0.87$; $p<0.001$; $df = 38$). This finding is taken as an indication for a close relationship between local production of inorganic phosphate and cementum formation rate. Although the cementum formation rate has not been measured directly, the thickness of the layer produced throughout life reflects, in a sense, its formation rate simply because cementum (under steady state conditions) is not degraded but gradually increases in thickness as time proceeds.

128 Expression of Alkaline Phosphatase mRNA in Developing Rat Molar by *in situ* Hybridisation. M.H. HELDER, J.H.M. WÖLTGENS*, A.L.J.J. BRONCKERS and D.M. LYARUU (Dept. Oral Cell Biol. ACTA, AMSTERDAM, Netherlands).

High alkaline phosphatase (AP-ase EC 1.3.1.3) activity has been associated for a long time with the cells of mineralizing tissues such as bone and teeth. By virtue of its high activity in calcifying tissues, the enzyme has for many years been implicated in mineralization processes. Histochemically high alkaline phosphatase activities are found in mineralizing molars in stratum intermedium and subodontoblastic layer against low activities in ameloblasts and odontoblasts. The aim of this investigation was to study by in situ hybridization the distribution of alkaline phosphatase mRNA in the developing rat molar tooth germ and correlate its localization with the enzyme activity observed histochemically. For this purpose unfixed frozen sections from 2 days old developing rat molars were incubated with ^{35}S-labelled riboprobes specific for rat bone alkaline phosphatase and counter-stained with haematoxylin. The sections incubated with antisense riboprobe showed a strong signal over the stratum intermedium and a diffuse signal over the stellate reticulum and dental pulp during darkfield and bright field illumination. These signals were not seen in control sections incubated with sense riboprobe. The alkaline phosphatase mRNA signal was found only in those cells which also exhibited enzyme activity histochemically. From these results we conclude that during tooth development the high alkaline phosphatase activity found histochemically in the stratum intermedium and subodontoblastic layer is a product of these cells.