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1687 **A Unifying Hypothesis For The Immunopathogenesis Of Oral Lichen Planus.**
P.B. SUGERMAN*, N.W. SAVAGE, L.J. WALSH and G.J. SEYMOUR
(Department Of Dentistry, The University Of Queensland, Brisbane, Australia).

The pathogenesis of oral lichen planus (OLP) remains largely unknown. Diverse exogenous agents including systemic medications, mechanical trauma, viral infection and contact sensitivity reactions appear able to trigger immunological disease mechanisms. Heat shock protein (HSP) may provide a link between these disparate aetiological factors. Accordingly, 34 sections of OLP and 14 sections of normal oral mucosa were stained with an anti-HSP antibody. The signal was amplified using the avidin-biotin complex and the color developed with diaminobenzidine. Microdensitometry demonstrated increased epithelial staining intensity in the OLP group ($p < 0.001$). Within the OLP group, 16 specimens (47%) exhibited enhanced staining in the basal cell layer. Lymphocytes were extracted from nine OLP lesions by collagenase digestion and expanded as long term T cell lines by repeated cycles of stimulation with phytohemagglutinin and rest in medium containing interleukin 2. In a standard tritiated thymidine uptake assay, these lymphocytes demonstrated significant proliferation when stimulated with purified protein derivative, of which HSP is a major constituent, with stimulation indices ranging from two to 132. Fifteen lesional T cell clones were isolated by limiting dilution and their ability to suppress concanavalin A (Con A)-stimulated proliferation of autologous lesional T cell lines was assessed. The majority of clones were CD3+CD4-CD8+αβ+ and functioned as suppressor cells. Peripheral blood lymphocytes from OLP patients responded significantly less than controls in both Con A-stimulated proliferation assays ($p < 0.001$) and Con A-induced suppressor assays ($p = 0.001$). These results are consistent with the hypothesis that, in OLP patients, diverse exogenous agents cause upregulated expression of HSP by oral mucosal keratinocytes. A reaction of cytotoxic T lymphocytes to these activated keratinocytes results in the tissue destruction which is characteristic of OLP lesions. Once initiated, such an immunological reaction may itself upregulate HSP expression by neighbouring keratinocytes, thereby resulting in lesion chronicity. Persistent suppressor activity of T cell clones within the lesion promotes chronicity. Individual susceptibility to OLP may result from dysregulated HSP gene expression by stressed oral keratinocytes or from defective suppression of an immune response following self HSP recognition. Philip Sugerman is supported by NH & MRC (Australia) Postgraduate Dental Research Scholarship.

1688 **Micromorphology of Resin/Dentin Interface (RDT) following a total etch technique in vivo F.R.TAY*, A.J.GWINNETT[1], K.M.PANG, S.H.Y.WEI (Faculty of Dentistry,Uni. of Hong Kong, [1]SUNY at Stony Brook, New York)**

This study investigated the micromorphological characteristics of the resin/dentin interface following a total etch technique in vivo. Class V preparations were made in 10 caries free, bicuspid teeth scheduled for extraction as part of an orthodontic treatment plan. They were prepared using ultra-high speed with water cooling. The surfaces of the preparations were conditioned for 20 sec with 10% phosphoric acid, washed out and briefly dried. All-Bond 2 primer and bonding agent (Bisco) was applied followed by incremental placement of Z100 (3M). After 25-35 days, the teeth were extracted, fixed in Karnovsky's solution and hemisectioned. One half of each specimen was demineralized, serial sectioned and stained for light microscopy. The remaining half was postfixed in osmium tetroxide, dehydrated in an ethanol series, embedded and prepared for SEM and TEM examination. A superficial zone of distinctive staining reaction occurred with Brown and Brenn and trichome stains. Measuring up to 8 μm, this zone was identified in the SEM as the hybrid layer. Resin was found in tubules in 75% of the remaining dentin and within 50 μm from the pulp. Solid cores of resin traversed the hybrid layer to become hollow with associated spherical bodies within the tubule compartment. These were identified in the TEM and the staining reaction indicated resin droplets continuous with a resin sheath which enveloped the intact odontoblast process. This study confirmed the presence of a hybrid layer in vivo. The ultrastructural relationship between resin and tissue components supported the existence of a sealed tissue interface following a total etch technique in vivo.

1689 **Identification of Mutans and Other Oral Streptococci with Arbitrarily Primed Polymerase Chain Reaction (AP-PCR).T.L. TRUONG*, C. MÉNARD, C. MOUTON and L. TRAHAN (GREB, Université Laval, Québec, CANADA).**

The identification and classification of the nonhemolytic or viridans group of streptococci have long been recognized as difficult and unsatisfactory. Phenotypic and genotypic heterogenity have resulted in ambiguous speciation, particularly with mutans streptococci and oral streptococci. This study was carried out to test the hypothesis that a recently developped technology, the arbitrarily primed-polymerase chain reaction (AP-PCR), could be used to identify and even classify oral and other streptococci. DNA was prepared and purified from 8 reference strains of *S. mutans*, 13 strains representing 9 oral streptoccocal species, a few unrelated species and from 49 fresh isolates of mutans streptococci from human saliva and dental plaque. DNA amplification was primed with one of three arbitrarily selected primers nine or ten nucleotides in length. The amplified DNA sequences (amplicons) obtained were compared by agarose gel electrophoresis. Species and strain specific fingerprints or randomly amplified DNA patterns (RAPD) were obtained not only from pure genomic DNA but also from the supernatants of crude cellular or colony extracts. The preliminary data demonstrated that AP-PCR could be used: (i) to distinguish the species *S. mutans* from other species of oral streptococci, (ii) to identify and possibly classify streptococci and (iii) as a valuable tool in bacterial epidemiology and transmission studies by virtue of its rapidity, efficiency and reproducibility in generating the genetic profiles of bacterial strains.
Supported by Canadian MRC grant MT-12077 and the Fonds Émile-Beaulieu.

1690 **High-Resolution Electron Microscopy of Crystals in Fluorotic Rat Enamel.** Y. YAMAGUCHI* and T. YANAGISAWA (Dep. of Ultrastructural Science, Tokyo Dental College, Chiba, JAPAN):

This study attempts to clarify changes caused by $F^-$ in enamel crystals from maxillary incisors of male Wistar rats given drinking water containing 0.044% NaF for 10 weeks. Enamel in the early maturation stage and immediately before eruption was observed.

Contact microradiographs of the enamel clearly revealed a highly mineralized layer extending from a point ½ of width of the surface and a low mineralized layer ⅔ of the way below it. Remarkably clear in the early maturation stage, these layers persist through the late maturation stage up to immediately before eruption. High-resolution electron microscopy showed crystals in the highly mineralized layer to be large and regularly shaped. Frequent excessive crystal growth and crystal-to-crystal fusion in this layer narrowed intercrystalline spaces. Crystals in the deep, low mineralized layer, however, were small. Irregularly shaped crystals were sometimes observed in wide intercrystalline spaces. Even shortly before eruption, none of the large crystals of the kind ordinarily observed in the deep layer of control-animal enamel was seen. These findings strongly suggest that NaF administration causes excessive crystal growth in the highly mineralized layer and the appearance of abnormally shaped crystals in the low mineralized layer. This work was supported in part by Grants-in-Aid for Scienc Research (JSPS Fellowships for Japanese Junior Scientists No.0895) from the Ministry of Education, Science and Culture, Japan.

1691 **Fractionation of Cell Attachment-Inhibiting Human Salivary Glycoprotein.** **A. ZENTNER* and T.G. HEANEY (Poliklinik für Kieferorthopädie, Mainz, FRG and Dental School, Liverpool, UK).**

**Initial attachment and locomotion of human gingival fibroblasts (HGF) are inhibited *in vitro* by a high molecular weight (HMW) sulphated glycoprotein isolate (SGP) derived from saliva (Heaney T.G., *J. Perio. Res.*, 1986). The aim of this work was to partially characterise the active agents in SGP and to determine whether all of its components are coadsorbed to hydroxyapatite (HA). SGP prepared by gel filtration on Sepharose CL-4B was subjected to ion exchange chromatography on DEAE Sepharose CL-6B and the 6 fractions (F1-F6) so obtained were further separated by SDS-PAGE. The latter showed that these contained mainly HMW substances, the most anionic fraction (F6) being composed entirely of material with a molecular weight in excess of 300kDa. All fractions possessed blood group activity, the sulphate concentrations of F1-F6 were 0.0, 1.6, 1.6, 2.3, 1.8 and 1.9μM respectively, and only F6 inhibited attachment of HGFs in an *in vitro* assay. SGP which had been adsorbed to HA and recovered by dissolution of HA and dialysis also inhibited HGF attachment and contained bands corresponding to F1-F6 as revealed by SDS-PAGE. It is concluded that the heterogeneous SGP is adsorbed qualitatively unchanged from saliva to HA, that its inhibition of HGF attachment depends only on its most anionic component which is presumed to be a HMW sulphated glycoprotein, and the activity of this agent is unrelated to presence of either SO, or blood group mojeties.**

1692 Adaptation of Masseter Intramuscular Connective Tissue Following Surgical Overloading. D. M. ABBOTT*, and P. C. DECHOW (Baylor College of Dentistry, Dallas, Texas, USA).

Little information exists about adaptations of the connective tissue architecture of the masticatory muscles during growth or in response to altered function. Such adaptations are important since they may effect muscle function and ultimately alter growth and form of the craniofacial skeleton and related tissues. In this study, we characterize the effects of surgically overloading the superficial (SM) and deep masseter (DM) muscles in rats by bilateral surgical ablation of a synergist, the temporalis muscle. Group A (N=9) served as surgical controls while Group B (N=9) underwent temporalis ablation. Following 8 weeks of healing, SM and DM of each rat were prepared for and observed with scanning electron microscopy. SM was divided into 10 regions for study, and DM into 12 regions. The density of the connective tissue and the collagen fibril diameters within each region were measured. Two-way ANOVA with posthoc Tukey tests was used to evaluate differences between sites and groups. In SM, there was no significant difference between groups, but the differences between sites were significant ($P<0.05$). In DM, significant differences occurred between both groups and sites. Differences were greatest in regions of muscle attachment to bone and aponeuroses, where Group B exhibited denser connective tissue and collagen fibrils of increased diameter. These results suggest that DM undergoes adaptational changes within the connective tissue architecture following surgical overloading by means of bilateral surgical ablation of temporalis. These adaptations may be compensatory due to the increased stresses placed upon DM by the loss of the synergistic effects of temporalis. Support: 1992 AADR Student Research Fellowship sponsored by 3M Health Care, and NIH Grant DE07761.

1693 Molecular Cloning of a Rat Submandibular Gland Apomucin. E.F. ALBONE*, F.K. HAGEN, B.C. VANWUYCKHUYSE and L.A. TABAK (University of Rochester, Rochester, NY USA).

A rat submandibular gland (RSMG) mucin was deglycosylated with trifluoromethane-sulfonic acid followed by periodate cleavage and β-elimination. Edman degradation of an endoproteinase Lys-C generated fragment revealed the amino acid sequence: (K)PTTD[A/S]TTPAPTTK. A degenerate oligonucleotide primer was designed from this sequence and was used to amplify a 400 bp product, which was employed to isolate four overlapping cDNAs from a RSMG cDNA library. The decoded cDNA sequence revealed a translated region of 966 nucleotides encoding a protein of 322 amino acid residues. The translational start site begins with a putative signal sequence comprising the initial 17 N-terminal residues. The predicted secreted portion of the apomucin revealed three distinct domains: an N-terminal domain which is enriched in Q (14%), P (13%), and Y (10%); a central region which consists of eleven, 39-base pair, tandem repeats with the consensus sequence PTTDSTTPAPTTK; and, a C-terminal domain which is enriched in T and S residues (47%) which are not part of a repeat motif. Northern blot analysis of RSMG RNA revealed at least two prominent transcripts which hybridized to the longest cDNA (1.5 kb, 10 kb). No hybridization signal was observed with RNA derived from rat liver, kidney, small intestine, stomach, lachrymal, or parotid glands. Collectively, our sequence and expression data indicate that the cloned RSMG apomucin is unlike any of the salivary (bovine, porcine, or human) or rat apomucins reported thus far. Supported, in part, by USPHS grant DE08108. E.F.A. was supported by USPHS grant T32 DE07202.

1694 **Tumor Necrosis Factor (TNF-α) Regulates the Expression of LPS Receptors on Human Periodontal Ligament Cells *in vitro*. T. A. BRADY, N.P. PIESCO, H.H. LANGKAMP, AND S. AGARWAL. (University of Pittsburgh, PA, USA).**

**Gingival fibroblasts (GF) are endotoxin (LPS) responsive cells which exhibit LPS receptors constitutively. We have shown that periodontal ligament (PDL) cells are unresponsive to LPS. However, when primed with TNF-α their response to LPS is similar to that of GF. The purpose of this study was to determine whether the TNF-α-induced modulation of PDL cell phenotype is mediated through the expression of LPS receptors. PDL cells were cloned and characterized by the presence of alkaline phosphatase and TGF-α specific mRNA. The presence of LPS receptors on PDL cells was determined by the binding of fluorescein-isothiocyanate (FITC) labelled LPS at 4° C. The responsiveness of PDL cells to LPS was assessed by expression of IL-8 specific mRNA by Northern blot analysis. PDL cells did not exhibit LPS receptors constitutively. However, treatment of PDL cells with rhTNF-α (500 pg/ml) for 5 days resulted in the expression of LPS receptors and IL-8 specific mRNA in response to LPS from *A. actinomycetemcomitans* (Aa) and *P. gingivalis* (Pg). The binding of LPS from *E. coli* ( 0, 1, 10, 100, 1000 ng/ml) to PDL cells was optimal at 100 ng/ml. The binding of FITC-LPS to PDL cells was LPS specific but not species specific, as it could be competitively inhibited by unlabeled LPS from *E. coli*, Aa or Pg. Characterization of surface proteins of PDL cells showed that the LPS receptor is distinct and it is synthesized following treatment of PDL cells with rhTNF-α. We demonstrate that rhTNF-α transiently modulates PDL cell phenotype and induces *de novo* synthesis of discrete receptors for LPS on PDL cells, and may play a significant role during inflammation by rendering PDL cells the ability to respond to a bacterial challenge.** **NIDR DEO 9830, DEO 7204.**