545 Smoking Exposure and Periodontal Disease. A Dose-Response Approach. J. BERGSTRÖM*, J. DOCK and S. ELIASSON (Karolinska Institute, Stockholm, Sweden)

Recent studies have provided evidence that tobacco smoking is a risk factor for periodontal disease. The present study was undertaken to further investigate whether or not a dose-response relation may exist between smoking and periodontal disease. The study was based on 258 individuals including 50 current and 61 former smokers. 100 of the individuals were prospectively followed up over 10 years. Smoking exposure was expressed as life-time exposure i.e. the product of number of cigarettes smoked per day and number of years of smoking. Periodontal pocketing and periodontal bone height were disease variables. Statistical significance was tested using paired and unpaired t-test after adjustment for age. The cross-sectional observations indicated that heavy smokers had more sites with pockets (Δ=18.5 t=2.88 P<0.01) and less bone height (Δ=6.3% t=3.56 P<0.001) than light smokers. Former heavy smokers had more sites with pockets (Δ=4.5 t=1.91 P=0.05) and less bone height (Δ=4.2% t=3.94 P<0.001) than former light smokers. The longitudinal observations indicated that heavy smokers developed more sites with pockets (Δ=15.0 vs 4.3) and lost more bone height (Δ=2.6% vs 2.1%) over time than light smokers. Both heavy and light former smokers exhibited a decrease in frequency of sites with pockets (Δ=-5.1 vs -5.2) and insignificant bone height reduction (Δ=1.4% vs 0.1%) over time and were no different from nonsmokers. These observations suggest that periodontal disease in terms of periodontal pocketing and periodontal bone height responds to smoking in a dose dependent way, further supporting the contention that tobacco smoking is a risk factor for periodontal disease.

546 Relationship of Calculus Status with Caries, Gingivitis and Salivary Factors K. PATTANAPORN* and J. M. NAVIA (Chiang Mai University, Thailand and University of Alabama at Birmingham, USA)

Dental calculus is a major oral health problem among Thai children. To investigate factors and disease interactions that contribute to calculus deposition, we studied in Thai children the relationship of dental calculus (calculus index; CI), with dental caries (DMFS, DMFT; WHO), gingivitis (gingival index; GI), plaque status (plaque index; PII), and the influence of selected salivary factors. A total of 439 children 11 to 13 years old were randomly selected from 18 schools in Chiang Mai, Thailand. Two hundred and six (206) children who had CI scores of ≥ 1.0 formed the calculus group, and the rest (233) children with CI scores of < 1.0 constituted the noncalculus group. Whole stimulated and unstimulated saliva, as well as stimulated parotid saliva, were collected from 60 children in each group and used to determine flow rate, pH and buffer capacity by the method of Ericsson and also by Dentobuff® strips. The data showed that mean±SD of CI, DMFS, GI, and PII for the calculus and noncalculus groups were 1.4±0 3 and 0.5±0.2; 2.1±2.8 and 2.0±2.6; 1.6±0.2 and 1.3±0.2; 2.0±0.4 and 1.8±0.4 respectively. Results indicated that calculus status was not significantly associated with caries (chi-square; p > 0.05) but significantly associated with gingivitis and plaque status (chi-square; p < 0.001). Mean flow rate, pH and buffer capacity of saliva from children in the two calculus groups did not differ significantly (ANOVA; p > 0.05). We conclude that calculus status and caries occurrence are independent events but calculus status was highly associated with gingivitis and plaque status. Salivary flow rate, pH, and buffer capacity do not by themselves contribute to calculus status in these Thai children. This study was supported by the John J. Sparkman Center, SPH/UAB, USA.

547 Influence of maternal periodontal status on colonization of suspected periodontopathogens in children. *E. KÖNÖNEN*, H. JOUSIMIES-SOMER, J. WOLF, S. ASIKAINEN* (National Public Health Inst., and Inst. of Dentistry, Univ. of Helsinki, Helsinki, Finland).

Children acquire oral bacteria via saliva from their frequent close contacts; the most important one is the mother. Her oral health status may have influence on the quality of transmitted species. Twenty-three children (mean age 32 mo, range 24-41 mo) were divided into 3 groups according to the maternal periodontal status determined by alveolar bone loss (AL) in panoramic radiographs, probing depth (PD) and bleeding on probing (BOP). Group I consisted of 10 children of periodontally healthy mothers (no AL, PD < 4 mm, BOP < 15%), Group II of 8 children of mothers with initial periodontitis (AL < 1/3 of the root length in < 30% of the surfaces, PD < 6 mm) and Group III of 5 children of mothers with moderate or severe periodontitis (AL ≥ 1/3 of the root length in > 30% of the surfaces, PD ≥ 4 mm). Stimulated saliva combined with a pooled bacterial sample from oral mucosal surfaces, and a pooled sample from 2 gingival crevices were collected from each child and cultivated on nonselective and selective media. *Actinobacillus actinomycetemcomitans* (*A a.*), *Porphyromonas gingivalis* (*P.g.*), *Prevotella intermedial/Prevotella nigrescens* (*P.i./P.n.*), *Fusobacterium nucleatum* (*F.n.*), *Selenomonas* spp., *Campylobacter rectus* (*C.r.*), *Eikenella corrodens* (*E.c.*), *Peptostreptococcus micros* (*P.m.*) and hemolytic phenotypes among gram-negative anaerobes were regarded as suspected periodontopathogens according to the literature. *A.a.* and *P.g.* were not found in any group; probably their colonization takes place later in life or their numbers remained below the detection level (10^2 CFU). The presence of *P.i./P.n.* in children increased in direct proportion to the worsening periodontal status of mothers, as 2/10 in Group I, 4/8 in Group II and 4/5 children in Group III harbored *P.i./P.n.* in the oral cavity. Interestingly, an atypical *P.i./P.n.* was recovered from half of the children in Group I and II, but none in Group III. The highest frequency of *C.r.* and *P.m.* was found in Group III children; 4/5 and 3/5 positive children, respectively. On the other hand, *F. n.* was present in all 23 children, and in addition, *Selenomonas* spp., *E. c.* and hemolytic phenotypes were equally found in all 3 groups. It is suggested that the colonization of certain periodontally "harmful" bacteria in children could be prevented As the periodontal health of child's close contacts seems to be of primary importance in achieving this goal preventive strategies should be directed to the source of infection.

548 Expression of *P. gingivalis* Fimbrillin in Vaccinia Virus. A. SHARMA,* S.J. RADEL, W.T. RUYECHAN[1] and R.J. GENCO (Departments of Oral Biology and Microbiology,[1] State University of New York at Buffalo, NY, USA).

Porphyromonas gingivalis is a pathogen strongly associated with some forms of adult periodontitis. Studies have indicated that *P. gingivalis* fimbrillin, a major 43 kDa subunit protein of fimbriae, confers immunity against *P. gingivalis* infection in gnotobiotic rats. Therefore, the identification of "critical" immunodominant epitopes of fimbrillin, their presentation to the host immune cell, and possible role in the induction of protective immunity are important prerequisites in understanding immunity to this virulence antigen. To this end, research in our laboratory has been directed towards delineating the antigenic domains of fimbrillin. In this report we describe the generation of recombinant vaccinia virus expressing fimbrillin that can be utilized for epitope mapping and development of vaccines. Briefly, the coding region of the *fimA* gene was cloned into the plasmid vector pSC11, downstream from the vaccinia virus promoter. The recombinant plasmid vector was then used to transform Vero cells infected with the wild type vaccinia virus. Recombinant vaccinia virus plaques were screened by hybridization with the *fimA* gene and purified. Purified, recombinant virus was then used to infect CV-1 cells, and total cell lysates were subjected to immunoblotting with anti-fimbrillin serum. A 43 kDa band that reacted with the anti-fimbrillin serum was detected following SDS-PAGE and immunoblotting. The results indicate that *P. gingivalis* fimbrillin can be expressed in mammalian cells using vaccinia virus. This recombinant vaccinia:fim virus can be utilized for epitope mapping or vaccine development. Supported in part by USPHS Grants DE08240, DE07034, and DE00518.

549 Oral *A. actinomycetemcomitans* in Young Men With Minimal Periodontal Disease. H.-P. MÜLLER*, L. ZÖLLER, T. EGER, S. HOFFMANN, D. LOBINSKY (University of Heidelberg; Ernst-Rodenwaldt-Institute, German Armed Forces, Koblenz; Germany).

A total of 1005 subgingival and extracrevicular samples from 201 male recruits, 18 to 25 years old, were selectively cultivated for *Actinobacillus actinomycetemcomitans*. The organism was isolated in 55 subjects (27%). 9.5% of pooled subgingival plaque samples from 1st molars, 14% cheek mucosa, 20% dorsum of tongue, and 20% saliva samples were culture-positive. In order to divide the study population into distinct clinical categories, cluster analysis was performed, based on previous caries experience, probing pocket depth categories, bleeding scores, visible plaque and calculus: 2 clusters (n=86 and n=92, respectively) were identified with no or minimal periodontal disease (mean±s.d. % of periodontal probing depth 1-2 mm 78.7±10.4% and 57.4± 12.6%, respectively; virtually no periodontal probing depth in excess of 4 mm) and a relatively low DMF-S (22±13). A 3rd cluster (n=22) had, in contrast, a high DMF-S (47.7±17.2) and a relatively high % of periodontal pockets of ≥5 mm (5.9±5.2%). Prevalence of *A. actinomycetemcomitans* in this cluster was 41%, while the organism was found in 23 and 27% in the minimally diseased populations (p<0.15). Whereas no heterogeneity of associations between subgingival and extracrevicular occurrence of the organism could be ascertained in different clusters, the organism was significantly more often identified in extracrevicular material, especially dorsum of tongue samples, as compared with subgingival plaque (McNemar's χ^2=12.45, p<0.001). Multiple linear regression analysis revealed the number of *A. actinomycetemcomitans* positive samples as well as the % of sites bleeding on probing being positively associated with the % of sites with a probing pocket depth of ≥5 mm (R^2=0.345, p<0.0001). The present large-scale investigation points to the wide distribution of this putative periodontopathogen in young individuals with minimal periodontal disease.

550 The Role of a Cell Surface Antigen of *Campylobacter rectus* in Phagocytic Killing K. OKUDA,*T.KIGURE, S.YAMADA, T.KANEKO, T.KATO and I.TAKAZOE (Departments of Microbiology and Periodontics, Tokyo Dental College, Chiba , Japan)

Campylobacter rectus has been implicated as an etiologic agent in adult periodontitis. The microorganism is often detected in large numbers in subgingival lesions of active sites. Bacterial surface characteristics have been shown to be associated with adherence to host tissues and resistance to phagocytic killing. Rabbit polyclonal antiserum to whole cells of *C.rectus* ATCC 33238 and monoclonal antibodies against the microorganism were prepared. An immunoglobulin 1 antibody reacted with the high-molecular-weight, 150 KDa, proteins from 4 of 9 *C.rectus* strains. Immunoelectron microscopic study showed the monoclonal antibody recognized the S-layer of *C.rectus* cells. It was found that phagocytic killing by pertoneal macrophages from guinea pig or human penferal leukocytes *in vitro* was significantly enhanced by the rabbit antiserum and moderately enhanced by monoclonal antibody against the S-layer, but was not enhanced by monoclonal antibody against lipopolysaccharide. However, neither rabbit antiserum nor monoclonal antibody showed any ability the antibody-complement mediated killing of *C.rectus* cells. The S-layer may be the factor resistant to antibody-complement mediated killing. Electron micrographs of macrophages and leukocytes exposed to viable cells of *C.rectus* in antibody-free culture showed injury to the phagocytic cells, indicating that the microorganisms possesses a leukocyte toxic activity. The rabbit antiserum and monoclonal antibody against S-layer partially inhibited the *C.rectus* attachment to G1 cells *in vitro*. The S-layer of *C.rectus* may be an evading factor from host defence mechanisms in periodontal lesions.
Supported by grant from Ministry of Education, Science and Culture of Japan, No.05454512

551 Prostaglandin E2 and Interleukin 6 in biopsies from the periodontal pockets. P.-Ö.SÖDER*, L. J. JIN, U. NEDLICH and B. SÖDER (Karolinska Institute, Stockholm, Sweden).

The study was to assess the levels of prostaglandin E2(PGE2) and Interleukin 6(IL6) in biopsies from deep periodontally involved sites and the presence of periodontopathogens from the sites. Thirty sites and biopsies were analyzed from 26 patients, 15 males and 11 females(39.5±3.2 years). The subjects were selected from 144 periodontal risk patients. They were periodontally treated and maintained every 6 months for 5 years and then designated to 2 well responding subjects(W.R.) 24 poor responding, divided in 10 localized periodontitis, LP 1, 8 localized periodontitis 2, LP 2, and 6 generalized periodontitis, GP, on basis of clinical criteria. Bacterial samples were taken from the sites before the biopsies and the presence of *Actinobacillus actinomycetemcomitans* , *A. a., Porphyromonas gingivalis, P.g.*, and *Prevotella intermedia, P.i.* were determined by cultivation. The biopsies were disintegrated and the PGE2 levels in supernatants were assayed by radioimmunoassay(^{125}I RIA Kit, NEN®) and IL 6 by ELISA. In biopsies from LP 1, LP 2 patients the PGE2 levels were 3.42±6.68 and 3.85±2.51 pg/mg wet weight respectively. In biopsies from GP patients PGE2 levels were 1.51±1.67 pg/mg and for W.R. patients 0.83±0.76 pg/mg wet weight. The levels of IL6 was in biopsies from LP 1 patients 7.15(Mean)(SE 2.5) and in LP 2 patients 1.31(Mean)(SE 0.4)pg/mg wet weight(p<0.05). The levels of IL6 was in biopsies from GP and W.R. patients were 4.75(Mean)(SE 2.0) and 0.44(Mean)(SE 0.44) respectively. In LP 1 patients *P.i.* was detected in 40 % and *P.g.* in 10 % of the sites. In LP 2 patients *P.i.* was found in 30 % and *A.a.* in 10 % of the sites and in GP patients *P.i.* was detected in 57.1 % of the sites.
Conclusions:The levels of PGE2 are high in localized forms and IL6 in certain forms of localized periodontitis. *Prevotella intermedia* is frequent in generalized periodontitis.
The study was supported by Swedish Patent Revenue Fund and the Swedish Dental Society.

552 Platelets in Human Crevicular Fluid: Interaction With Other Inflammatory Cells. A.D.STEINBERG*, M. ALVES, L.B.SILVERGLADE & T. JOINER. (U. of Illinois at Chicago, College of Dentistry, USA).

Platelets may be involved in the inflammatory process through the release of a variety of factors that could contribute to the amplification of the inflammatory process at a site of tissue damage. In a previous study Steinberg et al. (J Dent.Res,68:915, #393, 1989) observed platelet specific activation product β-thromboglobulin in crevicular fluid (CF) and appeared to increase with gingival inflammation. The purpose of this study was to identify the presence of platelets o their remnants in CF. Wash samples were obtained from gingival crevicular sites with a Loe and Silness gingival index of 0 to 3 and placed on glass slides. To identify the presence of platelet membranes a rapid immunocytochemical staining procedure combined with a high affinity primary antibody (mouse anti-human plate glycoprotein IIb/IIIa, CD41) were used (Dako Corporation). These samples were counterstained with Hemotoxylin to aid in light microscopic identification of other cells A positive staining for platelet membrane was observed in the samples. Furthermore, a close association was frequently observed between the positive platelet membrane staining and various inflammatory cells. Data suggest that platelet activation in CF and interaction with other inflammatory cells may induce th release of factors that could contribute to local inflammatory response associated with the destruction of periodontal tissues.
Supported by the G. Matula Fund, University of Ill at Chicago.