553

DNA Probe-Detected Periodontopathogens at Active Periodontitis Sites.
LJ. JIN*, P.-Ö. SÖDER, U. NEDLICH and B. SÖDER.
(Karolinska Institute, Stockholm, Sweden).

The prospective investigation was to determine the association between the presence and level of 7 subgingival periodontopathogens and attachment loss in advanced periodontitis sites, by using species-specific DNA probes, e.g. *A. actinomycetemcomitans (A.a.), P. gingivalis (P.g.), P. intermedia (P.i.), T. denticola (T.d.), E. corrodens (E.c.), F. nucleatum (F.n.) and C.rectus (C.r.).* The participants were advanced periodontitis patients who had been regularly maintained for 5 years after intensive treatment. Following baseline examination, 40 sites with probing depth ≥ 5 mm were sampled, and they were then monitored every 3 months without treatment until an active site was detected. These active sites showed a significant attachment loss at 6 months. Subgingival plaque samples were taken immediately from these active sites, and also from the matched inactive sites as controls. All 7 periodontopathogens were found at the detectable level of 6.0×10^3 cells in the 24 active and 16 inactive sites, and the positive frequency distribution of these bacterial species was as follows (Active / Inactive sites): *A.a.*, 29.2% / 12.5% ; *P.g.*, 45.8% / 43.8%; *P.i.*, 66.7% / 62.5%; *T.d.*, 62.5% / 62.5%; *E.c.*, 91.7% / 81.3%; *F.n.*, 83.3% / 68.8%; and *C.r.* 54.2% / 50.0%. 4.2% of the active sites and 18.8% of the inactive sites were without any of the 7 species. For the relative amounts of bacteria, *T.d.* significantly increased at the active sites (18.2±2.3%), compared to the inactive sites (9.9±2.8%) (p=0.0305). With respect to the retrospective microbiological data at baseline, the positive frequency distribution was as follows (Active / Inactive sites): *A.a*, 20.8% / 6.3% ; *P.g.*, 41.7% / 43.8%; *P.i.*, 66.7% / 56.3%; *T.d.*, 66.7% / 50.0%; *E.c.*, 62.5% / 68.8%; *F.n.*, 70.8% / 81.3%; and *C.r.* 62.5% / 62.5%. No significant difference was noted for the relative amounts of bacteria between the sites. The risk for disease progression at severe periodontitis sites seems to be related to certain periodontopathogens both qualitatively and quantitatively. This study was supported by the Lederle International, American Cyanamide Co. and the Swedish Institute (Grant No. 304/01).

554

Experimental Periodontitis in Non-insulin Dependent Diabetic Rats.
N. YOSHINARI*, M. OHHARA, T. TAKADA, K. INAGAKI, M. SUZUKI, E. MAK
and T. NOGUCHI. (School of Dentistry, Aichi-Gakuin University, Nagoya, Japan)

The purpose of the present study was to investigate the effect of a high carbohydrate diet and a sucr
solution on the periodontal lesion of spontaneous non-insulin dependent diabetes mellitus (NIDDM
rats, which were produced by the method of Goto and Kakisaki (GK), histologically a
histometrically. Forty five wistar rats as control and the same numbers of GK rats were included in
study. In the maxillary right side of every rats, a nylon thread was inserted into the interdental
between the right maxillary first and second molars to induce gingival inflammation. Then
animals were divided into three groups of wistar rats (J-1, J-2, J-3) and GK rats (G-1, G-2, G-
Groups J-1 and G-1, Groups J-2 and G-2, and Groups J-3 and G-3 received a normal laboratory di
the high carbohydrate diet, and a normal laboratory diet with 30% sucrose solution, respectively.
rats in each group were sacrificed at 6, 9 and 12 weeks. The blood glucose values increa
succesively from 3 to 12 weeks in Groups G-2 and G-3. Alveolar bone resorption of Groups G-2 an
G-3 was severer than that of Groups J-2 and J-3 at 12 weeks. The result of this study indicated that
metabolic control of NIDDM changed the periodontal condition in rats. This study was supported
the aid for Fundamental Scientific Research from Education Ministry, Grant 06771781.

555

Antigenic Heterogeneity of *Porphyromonas gingivalis* Fimbriae. J.-Y. LEE,* H.T. SOJAR, A. AMANO and R.J. GENCO (Institute of Oral Biology, Kyung Hee University, Korea and Department of Oral Biology, SUNY at Buffalo, USA).

Binding of *Porphyromonas gingivalis (Pg)* to pellicle-coated teeth is mediated to a large extent by fimbrillin, a major fimbrial protein. Antigenic heterogeneity of major fimbrial proteins among *Pg* strains was examined. Major fimbrial proteins were purified to homogeneity from *Pg* strains 2561, A7A1-28, and 9-14K-1 which are representatives of fimbrial groups tentatively defined by N-terminal amino acid sequences of fimbrillin molecules. Polyclonal antibodies were raised against purified fimbrial protein, and the IgG antibodies were purified. Antigenic heterogeneity of different major fimbrial proteins was examined by immunoblot analysis using IgG antibodies to purified fimbriae and as antigens; dissociated fimbriae from 24 strains of *Pg*. Fimbrial proteins of strains with amino acids V-G-D at positions 4 to 6 reacted strongly with antiserum to strain 2561 fimbriae (anti-2561) and weakly with anti-A7A1-28. Strains which belong to fimbrial groups with the amino acid residue E at position 4, and N-A-G at positions 4 to 6, reacted with anti-A7A1-28. In contrast, strains 9-14K-1 and HG 564 which have N-terminal sequences distinct from all other strains, and strains W50, W83, and AJW5 which are sparsely fimbriated, reacted only with anti-9-14K-1. Antigenic diversity in the major fimbrial proteins of *Pg* was demonstrated. Most of the strains belonging to the same fimbrial group, based upon N-terminal sequence of fimbrillin, appeared to be antigenically homologous. Finally, most of the strains tested belong to one of three antigenic groups based upon major fimbrial proteins. This study was supported in part by USPHS Grant Nos. DE08240, DE07034, DE04898, and DE06514.

556

Pathogen-related Oral Spirochaetes (PROS) in the Klipfontein population.
C.W.J. AFRICA[1]*, G. RIVIERE[2], J. REDDY[3] ([1]UWC Cape Town, [2]OHSU Oregon, USA, [3]UDW Durban S.A.)

The purpose of this investigation was to examine subgingival plaque from the periodontitis-resistant population of Klipfontein for the presence of PROS and *Treponema denticola* using mAbs.

Smears were made on microscope slides, fixed in methanol, blocked in 0.2% bovine serum albumin, 20% sucrose and 20 mM $Mgcl_2$ in TRIS-saline, then incubated successively with mAb, biotinylated goat anti-mouse IgG, streptavidin-alkaline phosphatase and finally, fast red substrate. Spirochaetes were evaluated by examination of 50 fields using phase-contrast/oil.

The results indicated that PROS was the predominating spirochaete, being present in 25 of the 68 samples examined, of which 6 had *T. denticola*. No spirochaetes were observed in 20 of the samples, while 21 had spirochaetes unreactive to either of the mAbs used. Only 2 had *T. denticola* in the absence of PROS. No relationship was observed between clinical status and the presence of PROS and/or *T. denticola* in these samples.

This study was supported by the MRC (S.A.).

557

Inflammatory cells subsets and episodic attachment loss. J. SATTLER* and U. ZAPPA
(University of Zürich, School of Dental Medicine, Switzerland)

The purpose of the present study was to assess possible associations between episodic probing attachment loss and immunohistochemically identified cell populations in periodontal lesions in humans. 20 systemically healthy adult patients with untreated advanced periodontitis were monitored during 10 months. At baseline and every month thereafter, probing attachment levels were measured using a pressure sensitive probe. Corresponding contralateral sites were identified, where 1 site had lost 2.5 mm or more attachment within the previous month (P-site), and the other site had not (NP-site). Biopsies were taken from these sites, processed and cut into 5 micron sections. The total number of cells was determined on sections stained with Hematoxylin and Eosin. Monoclonal antibodies were used to stain B-lymphocytes (CD 45 R), T-lymphocytes (UCHL 1), macrophages (MAC 387) and Langerhans cells (S-100). Polyclonal antibodies were used to stain all leukocytes (LCA) and plasma cells (Ig A, D, E, G, M). Cell counts were performed in standard areas at the apical portion of the junctional epithelium. Wilcoxon signed rank tests were used to assess differences between P and NP-sites. P-sites had statistically significantly more inflammatory cells than NP-sites ($p < 0.04$). P-sites had also significantly more LCA cells ($p = 0.01$), MAC-staining cells ($p = 0.03$), Ig A cells ($p = 0.03$) and Ig M cells ($p = 0.04$). High cell counts were present in a greater area in P-sites as compared to NP-sites for total cells, MAC and Ig A ($p < 0.05$). Clinically assessed episodes of disease progression seem to be associated with site specific shifts in total counts and individual cell populations. Supported by Swiss National Science Foundation Grant Nr. 31 337 and 3231-025 141.

558

Effects of Periodontal Therapy on Local Immune Response.
J.W. KLEINFELDER*, D.E. LANGE, W. BÖCKER. Dept. of Periodontology and Institute of Pathology, University of Münster, Germany

The host defence response is to be changed after periodontal therapy what should be studied in 10 patients with advanced periodontal disease at the age of 45 to 55.
We assessed probing-depth (PD), attachment level (AL) as well as bleeding, gingival and plaque indices. Both prior to therapy (group A) and 6 weeks after subgingival scaling (B) biopsies were performed at two lesions of 6 to 7 mm PD in order to identify the immunocompetent cells (CD 20, CD 30, CD 45, L1-antigen) and the immunoglobulines A, G and M by monoclonal antibodies using the APAAP-staining method. The cell density within the tissue sections was evaluated semiquantitatively (score 1 to 4). 6 weeks after an antibiotic regimen with 1 g tetracycline for 14 days we again biopsized two lesions (group C) as well as such that had obtained an additional scaling (D).
Mean PD of 6.3 mm, resp. AL of 7.5 mm revealed alterations of 1.1 (0.7) mm after scaling of 1.7 (1.1) mm after antibiosis as well as of 2.7 (1.8) mm after combined therapy. In the inflammatory periodontal lesions (group A) considerable infiltrations of plasma cells (score 3 and 4 with 74%) as well as of T-cells (47%) could be seen which were reduced to 47% (B), 17% (C), and 16% (D), resp. to 24% (B), 17% (C), and 11% (D). Ig-carrying cells were found in distinct conglomerations - score 2 and 3 with 44% (A), 10% (B), 18% (C), and 7% (D), whereas only isolated B-cells and PMNs could be detected: score 2 and 3 with 16% (A), 12%, (B), 11% (C), and 5% (D), resp. 11% (A), 18% (B), 0% (C), and 0% (D).
The clinical effectiveness of periodontal treatment corresponds clearly with a reduction of the immunocompetent cells; however, a slight inflammatory infiltration will always remain even in clinically healthy sites.

559

Molecular Genetic Analysis of Papillon Lefevre Syndrome. TC HART, A STABHOLTZ*, TE VAN DYKE, G BROACHA, L SHAPIRA, A ZLOTOGORSKY, A SOSKOLNE (Bowman Gray School of Med., NC, Hebraw Univ.-Hadassah, Israel, and Eastman Dental Cntr., NY)

Papillon Lefevre syndrome (PLS) is characterized by palmar plantar keratosis (PPK) and severe, early onset periodontitis affecting both deciduous and permanent dentitions. The association of severe, early onset periodontitis has historically distinguished the Papillon Lefevre syndrome from other more common forms of PPK. It has been hypothesized that individuals with PLS may have the same gene mutation as found in other forms of PPK, but have periodontitis due to a secondary infection by specific periodontopathic bacteria (e.g. *Actinobacillus actinomycetemcomitans*). Recently, several disorders involving palmar plantar keratosis have been mapped to the two families of cytokeratin genes on chromosomes 12 and 17. To test the generality of this finding, a linkage analysis was conducted between the Papillon Lefevre syndrome phenotype and highly polymorphic microsatellite markers in the chromosomal region of the cytokeratin family of genes. Fifty-one individuals (including 12 individuals affected with PLS) from an extended kindred segregating for the PLS received dental and dermatological examinations to determine PLS status. Genomic DNA was extracted from white blood cells and each individual was genotyped for the keratin gene markers KR9T, D12S137 and THRA1 using PCR, electrophoresis and autoradiography. Pairwise lod scores were calculated using the program Mendel. Results exclude linkage between the Papillon Lefevre syndrome and both the type I keratin gene cluster on chromosome 17q and the type II keratin gene cluster on chromosome 12q. These findings suggest that PLS is genetically distinct from other more common forms of PPK and suggest that mutations in genes other than keratin genes are responsible. Supported by DE10563

560

A case study of Papillon-Lefevre Syndrome: A treatment plan based on microbiological and serological investigations R.I. MARSHALL, R.P WIDMER, A.C CAMERON AND J.GOUVOUSSIS. (Westmead Hospital Dental Clinical School, Sydney, Australia)

Papillon-Lefevre Syndrome (PLS) is characterised by palmoplantar hyperkeratosis and severe prepubertal periodontitis affecting the primary and permanent dentitions. PLS appears to be inherited via an autosomal recessive gene (of homozygous transmission) with a prevalence of 1-4 per million of the population. No consistent serological defect or microbial profile has been described. In this study microbial and serological assays were performed on a 3 year old PLS patient and her non consanguineous parents. The mother had clinical and radiographic signs of rapidly progressing periodontitis, the father didn't. Pooled subgingival plaque samples were taken for DNA probe and direct ELISA identification and quantification and cultural identification of putative periodontal pathogens including *Porphyromonas gingivalis, Prevotella intermedia, Fusobacterium nucleanum and Actinobacillus actinomyceemcomitans* from each family member. Indirect ELISA against a panel of periodontal and non periodontal bacteria were used to develop total serum antibody and specific IgG profiles for each patient. The microbial results indicated a similar prevalence of test pathogens for the mother and child but not the father. As well the total serum and IgG profiles showed remarkable agreement between the mother and child but not the father. It is suggested that the periopathogens in the PLS patient are maternally derived. A treatment plan has been proposed based on treatment for the mother with conservative and antibiotic therapies. For the child, palliative care is planned until approximately six months prior to the eruption of the first permanent teeth, when any remaining primary teeth will be removed. Further microbial and serological screening is needed to ensure that the permanent teeth erupt into a pathogen free mouth with minimal risk of reinfection from exogenous sources. This case study indicates a possible pathogenic transmission from mother to sibling resulting in different manifestations of periodontitis due to differing host responses, and outlines a rationale for treatment.