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937

*Treponema denticola* Adherence to Cultured Periodontal Ligament and Endothelial Cells. H. AL-DERHALLI\*, E. MALDONADO, and D. THOMAS (Univ. Texas Health Science Center at San Antonio, Texas, USA)

In periodontal disease bacteria exert direct effects and trigger inflammatory host responses which cause loss of collagen attachment of tooth to bone, loss of bone, and eventual tooth loss. Spirochetes found in the periodontal pockets are implicated in the development of periodontitis. Oral spirochetes have been found in epithelial intercellular spaces, in connective tissue, in direct contact with the alveolar bone, and associated with microvessels. The purpose of this investigation was to study the binding of *T. denticola* to periodontal ligament (PDL) and endothelial cells. Understanding the adhesion-receptor interactions in a bacterial infection can lead to development of strategies to interrupt or prevent the disease process. Host cells were seeded into multiwell plates, allowed to form confluent monolayers, and spirochetes were added to the monolayers. Following incubation, the ability of *T. denticola* to adhere to host cells was assessed using an ELISA technique. All three *T. denticola* strains tested bound to both cell types, although *T. denticola* strain GM-1 bound to greatest degree to each cell type tested. Optimum parameters of binding, such as incubation time (three hours), bacterial number (approx.  $5 \times 10^7$  bacteria), and temperature ( $37^\circ\text{C}$ ) were determined using both host cell types. When the adherence was tested at optimum conditions, bacteria bound to greater extent to PDL cells ( $4.1 \pm 0.3\%$ ) than to gingival fibroblasts or keratinocytes ( $2.6 \pm 0.2\%$  and  $3.4 \pm 0.3\%$  respectively). Spirochete proteins which bound to host cells were identified by exposing detergent-extracted outer membrane material to host cell monolayers, washing the host cells, and solubilizing the cells (with adherent spirochete proteins). Immunoblots of this material indicated that bacterial proteins of 96, 72, and 63 kDa bound to PDL cells. These studies indicate that *T. denticola* bind specifically to PDL and endothelial cells, and that proteins of 96, 72, and 63 kDa may serve as adhesins for the binding to PDL cells.

938

Protective Effect against *P. g.* LPS-induced TNF-Mediated Skin Necrosis in Mice Lacking TNFR1 but not in Mice lacking TNFR2. S. AMAR\*, T. E. VAN DYKE, and H. BLÜTHMANN. (Boston University, CABR, Boston, MA, USA and Hoffman La Roche, Switzerland).

Tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ) is a central mediator of immune and inflammatory responses. Its activities have been shown to be mediated by two distinct receptors, TNFR1(p55) and TNFR2 (p75). The cytoplasmic domains of the TNFRs are unrelated suggesting that they link to different intracellular signaling pathways. Whereas most TNF responses have been assigned to one or the other of the TNF receptors (mostly TNFR1), there is no generally accepted model for the physiologic role of the two receptor types. To determine the nature and the role of the TNF- $\alpha$  receptor involved in *Porphyromonas gingivalis* (*P. g.*) LPS and TNF- $\alpha$ -induced skin necrosis, TNFR1-, TNFR2- and TNFR1/TNFR2-deficient mice were used. Skin abscesses were experimentally induced with local application of TNF- $\alpha$  or *P. g.* LPS. Wild-type and mutant mice (TNFR1, TNFR2 and TNFR1/TNFR2) were both injected subcutaneously with  $3\mu\text{g}$  of TNF- $\alpha$  for 5 days or received a single subcutaneous injection of *P. g.* LPS and examined 5 days later. Large macroscopic ulcerations were observed in TNF- $\alpha$  and *P. g.* LPS injected wild-type animals and in TNFR2-deficient mice while TNFR1-deficient mice did not exhibit any ulceration. Histological analysis of the biopsies revealed that *P. g.* LPS and TNF- $\alpha$  induced a similar pattern of tissue destruction which extended deep in the dermis whether in wild-type animals or in TNFR2-deficient mice. An intense immune infiltrate mainly composed of lymphocytes and neutrophils was observed in the wild-type animals and in TNFR2-deficient mice, while TNFR1-deficient mice were very mildly affected. TNFR1/TNFR2-deficient mice exhibited a similar immunity to TNF or LPS that TNFR1 mutants mainly due to the lack of TNFR1. The present data strongly suggest that TNFR1 rather than TNFR2 is engaged in *P. g.* LPS and TNF- $\alpha$ -induced skin necrosis and highlight the predominant role played by TNF- $\alpha$  in *P. g.* LPS induced inflammatory diseases. A role of TNFR2 in TNF-dependent homeostatic events is suggested (Supported by NIH/NIDR Grant DE10709).

939

Biological effects of scaling and root planning. I. Clinical parameters. M.A. CUGINI\*, A.D. HAFFAJEE, S. DIBART, R.L. KENT Jr. and S.S. SOCRANSKY, Forsyth Dental Center, Boston MA.

Investigations were initiated to examine the effect of different therapies on clinical and microbial parameters in subjects with periodontitis. Scaling and root planning (SRP) is a common form of periodontal treatment, yet little is known concerning the biological basis of its efficacy. The purpose of the present investigation was to examine the effect of SRP on clinical parameters in periodontitis subjects. 45 subjects with adult periodontitis (mean age  $47 \pm 11$  years) were monitored clinically and microbiologically prior to and 3 months post SRP. Clinical assessments of plaque, redness, suppuration, BOP, pocket depth and attachment level were made at 6 sites per tooth. Attachment level measurements were repeated at each visit and differences in means between visits used to assess change. Each subject received full mouth SRP under local anesthesia. Clinical data were averaged within each subject and then averaged across subjects for each visit. Differences in clinical parameters before and after SRP were sought using the Wilcoxon signed ranks test.

Mean pocket depth was significantly decreased from pre-therapy ( $3.30 \pm 0.06$  mm, Mean  $\pm$  SEM) to 3 months post-therapy ( $3.15 \pm 0.05$  mm). Attachment level, bleeding on probing, redness and suppuration also decreased but not significantly. Sites were subset on the basis of pre-therapy pocket depth. 0-3 mm pockets showed a non-significant increase in pocket depth ( $2.52 \pm 0.02$  to  $2.58 \pm 0.03$  mm) and attachment level ( $2.33 \pm 0.15$  to  $2.40 \pm 0.15$  mm) post therapy. 4-6 mm pockets showed a significant decrease in pocket depth ( $4.43 \pm 0.04$  to  $3.94 \pm 0.06$ ) and a non-significant gain in attachment post-therapy ( $3.89 \pm 0.26$  to  $3.47 \pm 0.22$ ).  $>6$  mm pockets showed a significant decrease in pocket depth ( $7.67 \pm 0.15$  to  $6.39 \pm 0.29$ ) and a significant gain in attachment level post-therapy ( $7.73 \pm 0.31$  to  $6.74 \pm 0.39$ ). Some subjects exhibited mean attachment loss post SRP. Thus, in accord with earlier studies, SRP appeared to maintain the status quo of the periodontium: improving moderate and deep sites, but contributing to a slight loss at shallow sites. Supported in part by NIH grant DE-04881.

940

Biological effects of scaling and root planning. II. Microbiological changes. A.D. HAFFAJEE\*, S. DIBART, M.A. CUGINI, C. SMITH, R.L. KENT Jr. and S.S. SOCRANSKY, Forsyth Dental Center, Boston MA.

While scaling and root planning (SRP) is often effective in controlling periodontal disease, its effects on the subgingival microbiota are poorly understood. The purpose of the present investigation was to examine the effect of SRP on the site prevalence of 40 subgingival species in subjects with adult periodontitis. Subgingival plaque samples were taken from the mesial aspect of each tooth in 41 subjects (mean age  $47 \pm 11$  years) prior to and 3 months post SRP. The presence and levels of 40 subgingival taxa were determined in 2,047 plaque samples using whole genomic DNA probes and checker-board DNA-DNA hybridization. Clinical assessments were made at 6 sites per tooth at each visit. The % of sites colonized by each species (prevalence) was computed for each subject at each visit. Differences in prevalence pre and post-therapy were sought using the Wilcoxon signed ranks test.

Overall, the mean prevalence of 4 species was significantly reduced after SRP: *P. gingivalis* ( $24 \pm 11\%$ ), *C. rectus* ( $17 \pm 11\%$ ), *B. forsythus* ( $46 \pm 5$  to  $26 \pm 3$ ) and *E. nodatum* ( $23 \pm 7$  to  $8 \pm 3$ ). Mean Simpson's Diversity Indices increased post SRP ( $0.10 \pm 0.02$  to  $0.16 \pm 0.04$ ), while the mean Evenness Indices decreased from  $1.64 \pm 0.09$  to  $1.26 \pm 0.03$  indicating a change in subgingival ecology. Suspected periodontal pathogens such as *P. intermedia*, *P. nigrescens*, *S. intermedium* and *A. actinomycetemcomitans* were minimally affected by SRP. Species that were significantly decreased by SRP showed proportionally greater reduction at shallow pockets (0-3mm) when compared with intermediate (4-6mm) or deep ( $>6$ mm) pockets. For example, the mean % of 0-3 mm pockets colonized by *P. gingivalis* was reduced from 17 to 7, while the prevalence at pockets 4-6 and  $>6$  mm changed from 30 to 15 and 49 to 31% respectively. *B. forsythus* was reduced at 0-3 mm pockets from 37 to 19%, from 55 to 34% at 4-6 mm pockets and 75 to 59% at  $>6$  mm pockets. Although SRP decreased the site prevalence of some (but not all) periodontal pathogens, it did not eliminate any species from a subject. SRP increased the prevalence of suspected beneficial species. Supported in part by NIH grant DE-04881.

941

Biological effects of scaling and root planning. III. Poor responders. S.S. SOCRANSKY\*, S. DIBART, M.A. CUGINI, C. SMITH, R.L. KENT, Jr. and A.D. HAFFAJEE, Forsyth Dental Center, Boston MA.

While most patients respond well to SRP, a subset exhibit mean attachment loss post therapy. Poor responders might harbor higher levels of periodontal pathogens than good responders. To test this hypothesis, 15 subjects who showed mean attachment loss after SRP were compared with 26 subjects who showed mean attachment level gain. Clinical assessments of plaque, redness, suppuration, BOP, pocket depth and attachment level were made at 6 sites per tooth. Attachment level measurements were repeated at each visit and differences in means between visits used to assess change. Microbiological assessment consisted of evaluation of the prevalence and levels of 40 subgingival taxa in subgingival plaque samples from the mesiobuccal site of each tooth (maximum 28 sites) in each subject. The prevalence of each species was computed for each subject and then averaged across subjects in the two treatment response groups. Differences between groups were sought using the Mann-Whitney test.

The hypothesis that periodontal pathogens were more prevalent in poor response subjects pre-therapy was rejected. For example, the % of sites colonized in good and poor response subjects respectively for *P. gingivalis* was  $29 \pm 5$ ,  $16 \pm 5$ ; *C. rectus*  $24 \pm 4$ ,  $5 \pm 2$ ; *B. forsythus*  $53 \pm 6$ ,  $36 \pm 7$ ; *P. intermedia*  $42 \pm 5$ ,  $39 \pm 8$ ; *T. denticola*  $39 \pm 5$ ,  $14 \pm 1$ . Suspected beneficial species were significantly more prevalent in good responders than poor responders prior to therapy; e.g. *A. viscosus*  $69 \pm 6$  vs.  $29 \pm 6$ ; *S. sanguis*  $44 \pm 5$  vs.  $23 \pm 6$ . The prevalence ratio of pathogen to beneficial species, pre-therapy differed in the 2 groups. For example, the *P. gingivalis*:*A. viscosus* ratio was  $0.59 \pm 0.15$  in good response subjects and  $1.79 \pm 0.89$  in subjects exhibiting a poor response. Other species that showed similar ratios with *A. viscosus* were *B. forsythus*, *P. nigrescens*, *F. nucleatum* subspecies, *S. intermedium* and *P. micros*. A poor response to SRP was not associated with higher levels of suspected pathogens pre-therapy, but lower levels of suspected beneficial species. The ratio of pathogen to beneficial species pre-therapy was a useful predictor of therapeutic outcome. Supported in part by NIH grant DE-04881.

942

Effect of Non-Surgical Treatment on Elimination of 7 Periodontopathogens in Refractory Periodontitis Patients. L. J. JIN\*, P.-Ö. SÖDER, and B. SÖDER. (Karolinska Institute, Stockholm, Sweden).

The aim of the study was to evaluate the elimination rate of 7 selected subgingival bacteria in periodontal pockets following scaling and root planning in patients with refractory periodontitis. 99 sites with probing depth  $\geq 5$  mm were selected from 10 refractory periodontitis patients (9-10 sites per subject). Following baseline clinical examination and bacterial sampling, all subjects received one-session of scaling and root planning. Clinical examination and bacterial sampling were repeated one month after the treatment. The presence of *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.) was determined by DNA probes with a minimum sensitivity of 6,000 cells/site. One month after the treatment, elimination rate for the 7 species was as follows, respectively: A.a., 72.4%; P.g., 64.3%; P.i., 47.6%; T.d., 29.3%; C.r., 20.0%; F.n., 13.2%; and E.c., 6.7%. For P.g. and T.d. lower elimination rate was found at the sites with deeper probing depths at baseline ( $p < 0.01$ ). For the sites free of one of the 7 species at baseline, the detection rate for respective species after treatment varied greatly: E.c., 70.8%; C.r., 52.9%; P.i., 47.2%; T.d., 39.0%; F.n., 34.8%; A.a., 15.7%; and P.g., 9.3% ( $p < 0.01$ ). For the sites free of all the 7 species at baseline, the detection rate also varied among the species: E.c., 53.5%; C.r., 23.1%; F.n., 23.1%; T.d., 15.4%; A.a., 15.4%; P.i., 7.7%; and P.g., 7.7% ( $p < 0.05$ ). The present study suggests that scaling and root planning alone has a limited effect on elimination of subgingival periodontopathogens in refractory periodontitis patients. In the sites initially free of the bacteria, a high incidence of colonizing rate may exist. The study was supported by the Swedish Institute, Swedish Patent Revenue Fund and the Karolinska Institute.

943

The Non-surgical Treatment of Periodontal Patients. J. GIORDANO,\* WJ LOESCHKE, S. SOHRREN, R. HUTCHINSON, CF RAU, L. WALSH, AND MA SCHORK, University of Detroit Mercy, University of Michigan, Ann Arbor, Michigan, USA

Previous double blind studies have shown that statistically significant improvements in clinical health could be observed in metronidazole-treated groups compared to placebo-treated groups who had received the normal standard of care. In the present investigation we extend these studies by asking the question as to how much surgery can be eliminated if the patients were retreated with systemic and/or locally delivered antimicrobials. No patient received more than two rounds of local treatment. All treatments were randomly assigned following a double-blind protocol. The reduction in surgical needs after the first round of treatment (scaling and root planning (S&RP) and either placebo, metronidazole or doxycycline) was significantly greater in the antimicrobial groups ( $p=0.03$ , ANOVA). 37% of the patients needed no further treatment and proceeded directly to the maintenance phase. Another 20% of patients proceeded to maintenance phase after individual teeth were treated with ethylcellulose films containing either placebo, 20% metronidazole, or 20% chlorhexidine. A second and third round of treatment with ethylcellulose films resulted in another 14% and 7% entering the maintenance phase of treatment. Overall, there was a 94% reduction in the need for periodontal surgery about individual teeth and an 81% reduction in the need for tooth extractions. Only 93 teeth out of an initial total of 783 teeth in 90 patients actually needed surgery or extractions. Eighty one percent of the patients entered the maintenance phase without needing periodontal surgery or extractions. These findings indicate that a treatment paradigm based upon the diagnosis and treatment of specific bacterial infections is likely to be successful for the majority of periodontal patients. Supported by NIDR grant DE06030.

944

Increase in Bleeding Pockets in Periodontal Patients During Supportive Therapy. V. MULLER-CAMPANILE\*, M.S. TONETTI, and N.P. LANG (School of Dental Medicine, University of Bern, Switzerland).

Periodontal maintenance is the most significant treatment phase for long-term therapeutic success. The aim of this survey was to study the long term effects of periodontal maintenance care in a University clinic in terms of increase in the number of periodontal pockets. 273 patients (aged  $52 \pm 14$  years, 41% males) were kept on recall for 671 months. In 256 patients the initial diagnosis was mild to severe periodontitis, while remaining had gingivitis. At the beginning of the maintenance period patients had 2c teeth, a full mouth bleeding score (FMBS) of  $21.6 \pm 15.5\%$ , and  $4.8 \pm 7.9$  bleeding pockets (mm). The increase in the number of pockets 4-5 mm deep was  $5.9 \pm 7.3$  in subjects v baseline diagnosis of gingivitis,  $5.8 \pm 8.4$  in the mild,  $8 \pm 13.4$  in the moderate and  $11.2 \pm 15$  severe periodontitis subjects. Similarly the increase in the number of bleeding pockets v  $3.2 \pm 5.3$ ,  $3.3 \pm 6.7$ ,  $4.6 \pm 9.9$ , and  $4.9 \pm 11.8$ , for the different initial diagnosis, respectively multivariate model was constructed predicting the increase in the number of bleed pockets as a function of initial diagnosis, type of treatment, smoking status, sex, age, FMI time on the recall program and number of teeth lost while on the recall program. Type treatment, initial and final FMBS, smoking status and time on recall program were significantly associated with the increase in number of bleeding pockets ( $P < 0.001$ , Squared  $Q = 29$ ). It is concluded that treatment approach, residual level of infection, smok status and the length of time on the recall program were significantly associated with increase in bleeding pockets in this treated and well maintained periodontal population. Supported by Swiss NF Grant #32-37763.93, and Clinical Research Foundation, Bern.