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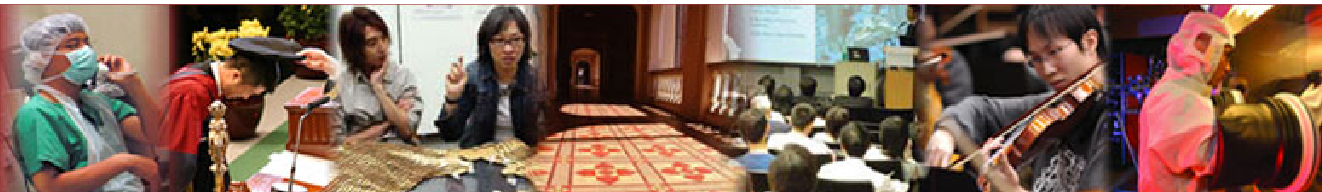

Title	Cytotoxic activity of Treponema denticola
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Citation	The 74th General Session and Exhibition of the International Association for Dental Research, San Francisco, CA., 13-17 March 1996. In Journal of Dental Research, 1996, v. 75 Sp Iss, p. 131, abstract no. 907
Issued Date	1996
URL	http://hdl.handle.net/10722/53856
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905 Treponema denticola Outer Membrane Modulates Calcium Responses in Human Gingival Fibroblasts K KO* M GLOGAUER C A G McCULLOCH and R P ELLEN (Department of Periodontics and MRC Group in Periodontal Physiology, Faculty of Dentistry University of Toronto Canada)

Treponema denticola (*Td*) binds to human gingival fibroblasts (HGFs) and perturbs physiological functions of their cytoskeleton The effects are mediated by whole bacteria and by components of *Td*'s outer membrane Our aim was to determine how an outer membrane extract (OM) alters HGF responses to physical stimuli We measured spontaneous oscillations of $[Ca^{2+}]_i$ in resting HGFs and transient $[Ca^{2+}]_i$ responses to plasma membrane stretching after pretreatment with OM of *Td* 35405 Collagen coated ferric oxide beads were bound to substrate attached HGFs and they were placed in a magnetic field to deliver controlled forces to their plasma membrane Single cell ratio fluorimetry of fura 2 loaded cells demonstrated a large increase in $[Ca^{2+}]_i$ in response to the stretch stimulus HGFs that had been incubated with OM for 30 min exhibited marked reductions in the stretch evoked $[Ca^{2+}]_i$ transients (142 +/ 22 % above baseline vs 330 +/ 63 % for controls, n=10 p=0 006) and marked reduction in the time to peak $[Ca^{2+}]_i$ (14 +/ 2 s vs 23 +/ 3 s for controls, p=0 017) Normal spontaneous $[Ca^{2+}]_i$ oscillations were eliminated by adding OM OM mimicked the effects of thapsigargin which blocked internal Ca^{2+} release of HGFs OM and the stretch activated Ca^{2+} channel blocker gadolinium chloride had additive suppressive effects on the stretch evoked $[Ca^{2+}]_i$ transients *T. denticola* outer membrane suppresses intracellular calcium responses to membrane stretching of fibroblasts by blocking internal calcium release. $[Ca^{2+}]_i$ responses coincide with F actin disruption and suppression of the inositol phosphate pathway reported elsewhere by us Supported by grant MT 5619 and a group grant from MRC Canada

906 The Velocity of *Treponema denticola* Cells J D RUBY*, P KHANDARE AND N W CHARON (West Virginia University, Morgantown, WVA).

We examined the translational velocity of *T. denticola* ATCC 33520 using dark-field video microscopy. Previous studies of *T denticola* ATCC 33520 motility (Pietrantonio, F et al 1988 Can. J. Microbiol 34 748-752) revealed the average speed to be 0 23 μm s^{-1} in methylcellulose (mc) having a viscosity of 9 57 centipoise (cP) at 25° C Wet mounts of *T denticola* cells were prepared anaerobically in NOS medium with 5% rabbit serum, and mc was added to determine the effect of viscosity on velocity The effect of temperature and glucose on cell velocity was also measured Velocities were determined by videotaping translating cells at 5500X and measuring the distance the cell traveled over a time period of 2-10 s. No translational activity was observed in liquid broth (cP = 0.87) However, as the viscosity of the medium increased, the velocity of *T denticola* cells increased while the temperature was held constant at 25° C, i.e., 1 47 ± 0 29 μm s^{-1} at 7.0 cP, 2 53 ± 0 34 μm s^{-1} at 9.2 cP, 4.20 ± 1 16 μm s^{-1} at 155 cP and 6.31 ± 1.25 μm s^{-1} at 216 cP Remarkably, as the temperature was increased to 35° C the cell velocity increased substantially, i.e , 5.48 ± 1.25 μm s^{-1} at 9.2 cP and 19 31 ± 4.46 μm s^{-1} at 140 cP (mean ± SD, n = 30). The presence of 100 mM glucose in the medium had no appreciable effect on cell velocity. We conclude that *T. denticola* cell velocity was increased as the medium became more viscous and elevated to 35° C, the addition of glucose did not effect cell velocity Supported by NIDR Grant DE00257.

907 Cytotoxic Activity of *Treponema denticola* W K LEUNG* P M HANNAM, V -J UITTO and B C McBRIDE (Dept of Microbiology and Immunology, University of British Columbia, Canada, and University of Hong Kong)

This study investigated the interaction of *T denticola* whole cells or its outer sheath with cultured eukaryotic cells Cultured porcine periodontal ligament epithelial cells (PLE) and various cell lines were challenged with *T.denticola* ATCC 35405 whole cells or with the major outer sheath protein (Msp) complex, which contains the Msp protein and an associated 95 kDa chymotrypsin like protease Msp complex was isolated from whole *T denticola* either by sonication or by extraction with 0 1% octyl-POE followed by concentration and dialysis to remove the detergent A modified ELISA assay showed that spirochetes adhered to glutaraldehyde-fixed PLE, and that pretreatment of *T denticola* with the protease inhibitors TPCK or PMSF blocked this binding and chymotrypsin-like activity Msp complex was cytotoxic to PLE cells and to to several cell lines as measured by the ability of cells to metabolize tetrazolium (MTT assay) or by quantitating lactic dehydrogenase released upon cell lysis (LDH assay) Cell changes were also observed by electron microscopy These included membrane disruption, vacuolization, and loss of cell contacts Oral treponemal strains varied in the size of their Msp peptide, in the amount of Msp protein produced, and in the cytotoxic effects of their Msp complexes We conclude that *T.denticola* ATCC 35405 is cytotoxic to a variety of epithelial cells and that the cytotoxicity is mediated by outer sheath components present in the Msp complex. This study was supported by the Medical Research Council of Canada and the Canadian Bacterial Diseases Network.

908 Expression of Chimeric Bone Sialoprotein Promoter-Luciferase Gene *in vivo*. J CHEN[1]*, H F THOMAS[1], J SODEK[2], H JIANG[1], H. JIN[1] ([1]Pediatric Dentistry, U of TX Hlth Sci Ctr San Antonio, TX, [2]U of Toronto, Toronto, Ont)

Bone sialoprotein (BSP) a major non collagenous protein, is a tissue specific glycoprotein found in mineralized connective tissues To determine the molecular basis for the tissue-specific and developmentally regulated expression of BSP gene, we have recently generated transgenic mouse lines in which the rat BSP gene promoter has been incorporated into the mouse genome A chimeric gene comprising 2 7 kb of the rat BSP 5'-flanking promoter fused with a firefly luciferase gene, was used in the microinjection To test for gene integration tail DNA from the offspring was digested with *Eco*R I and hybridized with [32P]-labeled construct DNA in Southern hybridization Positive founders were bred with normal mice and the positive progenies were identified by polymerase chain reaction and further confirmed by Southern hybridization Various bone tissues from neonatal transgenic mice (line #14) as well as from normal littermates at different developmental stages were dissected and used for RNA isolation and subsequent Northern hybridization analysis using [32P]-labeled luciferase gene and rat BSP cDNA, respectively Bone tissues such as calvariae, tibiae and mandibles from transgenic mice expressed luciferase mRNA at 1 day after birth and reached the maximal level at day 6 The intensity of luciferase mRNA decreased afterwards and was barely detected in the tissues from 20-day-old mice, corresponding to the endogenous BSP gene. Immunohistochemical studies using a polyclonal antibody against luciferase demonstrated that, in 6-day old transgenic mice, strong immunostaining was seen in differentiated osteoblasts, bone matrix, odontoblasts, predentin and dentinal tubules Some hypertrophic cartilage cells were also positive with the luciferase antibody These results demonstrate that the 2.7 kb BSP promoter directs the transcription of luciferase gene in a manner consistent with the tissue-specific and developmental regulation of the BSP gene. Supported by NIH/NIDR Grant R29DE11088 and in part by Smokeless Tobacco Research Council Grant 0472

909 Confocal Imaging of NonCollagenous Bone Proteins in UMR 106.01-BSP Cultures. J QUINN*, J MARTIN and C STANFORD (Dows Institute for Dental Research and Deptartment of Orthopaedic Surgery, University of Iowa, IA 52242)

In vitro mineralization of the UMR 106 01 BSP cell line demonstrates a rapid, phosphate-dependent bio-apatitic mineral deposition In turn, bone sialoprotein (BSP) expression may play a role in this culture phenotype The purpose of this study was to image changes in the location and immunolabeling intensity of BSP, osteopontin (OPN) and Alkaline Phosphatase (AP) epitopes under mineralizing (5mM ß-Glycerophosphate, ß-GP) and non-mineralizing conditions UMR cultures grown in Permanox slide 4 chamber wells (0 5 million cells per well) 24h (preconfluent) or to 72h in EMEM + 10% FCS (nonessential amino acids + 20mM HEPES), pre-fixed and permeabilized (20% DMSO in MeOH),washed and incubated 1h (25°C) with undiluted monoclonal primary antibody (WVID1) for BSP, (MDIII) for OPN and (B3-50) for AP (Dev Studies Hydridoma Bank) Following through washing (0 02M PBS), 1/300 FITC Goat anti-mouse Ab was added (1h, 25°C), washed and z-axis reconstructive confocal fluorescent imaging performed (MRC600, BioRad) **Results.** UMR cultures demonstrated an intense localized paranuclear labeling for BSP associated with the golgi apparatus Addition of ß-GP in the last 24h appeared to enhanced the intensity of BSP labeling in the cell layer Labeling for OPN in preconfluent cultures demonstrates low levels of diffuse intracellular labeling which became elevated by day 3 of culture Imaging of AP suggested a generalized diffuse labeling that was not altered with the formation of mineral deposition These results suggest localization of BSP and OPN appear to occur in a time and density dependent manner. NIH P30DE10126, Roy J Charitable Trust, DERC, Implant Dentistry Research and Education Foundation

910 Loss of BSP Expression Correlates with loss of Biomineralization. C STANFORD*, K HOLTMAN, J MARTIN, J STEVENS and R MIDURA (Dows Institute and Department of Orthopaedic Surgery, Univ of Iowa, USA)

Bone Sialoprotein (BSP) expression by the UMR 106 01 BSP bone cell line may correlate with the rapid biomineralization response described within the first 12 to 15 hours of culture (JBC 270 9420-9428,1995) By dilution analysis, a non mineralizing cell type (referred to as 'UMR-UI') was isolated from the parental UMR 106 01 BSP line The purpose of this study was to evaluate different bone-associated properties of the UMR-UI cell type Cultures (2000 cell/mm²) were grown (EMEM + 10% FCS, 20mM HEPES, gentamycin) for 48 h followed by 24 h supplementation with or without organophosphate (ß-GP) or P_i (0-10mM) followed by atomic absorption for $[Ca^{++}]$ in the cell layer Cultures were also metabolically labeled for BSP and proteoglycans with SO_4 free Media 199 + 5 % FCS (100 μCi/ml [35S], 50 μCi/ml [3H]glucosamine) during the last 24 h of culture, followed by guanidine extraction of the cell layer and media, G-50, Q-Sepharose and Superose-6 FPLC BSP was identified through Western blotting **Results** UMR UI cells demonstrated a lack of biomineralization upon exposure to either ß-GP or inorganic phosphate. Morphologically, UMR-UI cells appeared as a uniform "spindle-shaped" population of cells which labeled with a diffuse cytoplasmic staining for BSP (WVID1 Mab with FITC Goat anti-mouse) but with an intense labeling for osteopontin (B3-50) in contrast to the UMR 106 01 BSP parental cell line upon Confocal microscopy While AP activity of UMR-UI was less than UMR-BSP (6 ± 0 5 versus 36 ± 5 μmol PNP/min/mg DNA n=12), studies with UMR-UI cells of the kinetic P_i hydrolysis from orangophosphates demonstrated complete hydrolysis by 12 hours UMR-UI cells did not demonstrate a $^{35}SO_4/^3H$ glucosamine BSP peak (identified by Western blotting with WVID1 Mab) on FPLC Northern analysis suggested a 10 fold lower level of BSP message (with a single 1 9 Kb band) in UMR-UI versus the parental line These results suggest the UMR UI cell type may be a model for clarifying the role of BSP in biomineralization Carver Charitable Trust NIH/NICH DNO1-HD-6-2915, NIH/NIDR P30 DE10126-04 and DE11170-02A1

911 Time dependent expression of low molecular BSP fragments in BMP induced ectopic osteogenesis D KOBAYASHI* M MIZUNO H TAKITA and Y KUBOKI (Sch of Dent Hokkaido Univ , Sapporo, Japan)

Bone sialoprotein is unique in bone and dentin but its precise roles in these tissues is still unknown although several hypothesis have been presented We chose BMP-induced ectopic chondro- and osteogenesis as a model system to examine the roles of this protein Partially purified bovine BMP obtained by three-step chromatographic procedure, which constanted all the active BMPs (natural BMP cocktail) was combined with insoluble bone matrix and implanted in rat subcutaneously Expression of BSP in the implants was followed by monoclonal antibody as previous reported Immunostaining studies showed that BSP localized in osteoblast lining new bone surface at 5 weeks Western blotting showed 53kDa and 30kDa bands instead of 57kDa band which normally found in rat femur These two fragments also metabolically labeled by [3H]proline Total amount of the fragments rapidly increased after three weeks, and at 5 weeks attained 3 times as high as that of 2weeks after implantation This time-dependent changes was almost parallel to that of osteocalcin Amount of bone estimated by calcium content increased until 3 weeks and kept plateau thereafter, and alkaline phosphatase activity was eminent only in the first 3 weeks It was concluded that the fragmented 53kDa and 30kDa BSP may played a role of maintenance or remodeling in the BMP induced ectopic bone formation.

912 Dentin Sialoprotein, Bone Sialoprotein, and Osteopontin Inhibit Hydroxyapatite Growth A L. BOSKEY*, L SPEVAK, and W T BUTLER (The Hospital for Special Surgery, New York, NY and Univ Texas-Houston, Dental Branch, Houston, TX)

The phosphorylated sialoproteins osteopontin (OPN) and dentin sialoprotein (DSP) inhibit growth and proliferation of apatite (HA) crystals in both gelatin and agar gels, while bone sialoprotein (BSP) acts as a nucleator in these systems In solution, BSP has recently been reported to inhibit apatite growth DSP, a 53kDa glycoprotein first isolated from rat dentin (Matrix 12 343,'92) is synthesized only by odontoblasts and certain pulp cells, and is secreted into mineralized dentin Although DSP is similar in its overall properties to OPN and BSP isolated from bone, its predicted sequence is unrelated to those for OPN and BSP (J Biol Chem 269 3690,'94) It is also has fewer phosphorylation sites than bone OPN Here we report the relative abilities of each of these proteins derived from rat bone or dentin to inhibit proliferation and growth of HA seed crystals in a gelatin gel system Each of the proteins inhibited growth when 0.5mg/ml HA seeds were included with the protein in the center of a gelatin gel into which Ca and P ions diffused from opposite sides Mineral accumulation was monitored at 5 days, and the yield in the presence of protein compared to that in control gels OPN was the most effective inhibitor of HA growth, with 33±2% inhibition at 25μg/ml At 25μg/ml BSP caused 28±3% inhibition, and DSP 15±1% Dephosphorylated OPN had no effect The phosphorylated sialoproteins thus may serve as both nucleators and crystal growth inhibitors, depending on concentration, extent of phosphorylation, and conformation.

Supported by NIH grants DE04141 and DE05092