Association of collagen-binding protein with the development of IgA nephropathy-like nephritis in rat model

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Background: We previously reported that administration of collagen-binding protein (Cnm)-positive *S. mutans* caused IgA nephropathy-like nephritis in the jugular vein of model rats. In the present study, the involvement of Cnm in development of IgA nephropathy-like nephritis in a jugular vein model was investigated using a Cnm deletion mutant strain and its complement strain.

Methods: An *S. mutans* SN74 strain, that with Cnm deletion, and a complement strain were used in this study. The tested strain was administered through the jugular vein of Sprague-Dawley rats (4-week-old males). After 45 days, the rats were euthanized, then urine and blood samples collected, and kidneys extracted. Renal tissue pathology in the kidneys was evaluated using periodic acid-schiff (PAS) staining and fluorescent immunostaining with IgA antibodies.

Results: There were no significant differences in major serum measurements or urinary protein levels among the groups. On the other hand, there was a greater rate of hematuria positivity in the SN74 and complement groups than in the deletion group. Mesangial proliferation scores, determined in images following PAS staining, for the SN74 and complement groups were significantly higher as compared to the deletion group ($P<0.05$, $P<0.001$, respectively). Furthermore, immunostaining evaluations demonstrated significantly greater levels of IgA deposition in the mesangial region in the SN74 and complement groups, and IgA positivity than in the deletion group ($P<0.05$, $P<0.01$,

respectively).

Conclusion: These results suggest that Cnm may be involved in IgA nephropathy-like nephritis in rats induced by administration of *S. mutan*s into the jugular vein.

Effects of Cyclodextran on Exfoliation of Biofilm Formed by *Streptococcus mutans*

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Background: Cyclodextran (CI), a cycloisomaltooligosaccharide, is known to function as a dextran analogue. In our previous report, CI was shown to have a strong inhibitory effect on glucosyltransferase B produced by *S. mutans*, a major pathogen of dental caries. The present study was conducted to investigate the effects of CI on exfoliation of biofilm formed by *S. mutans*.

Methods: *S. mutans* MT8148 bacteria were stained with SYTO® 9 and suspended using chemically defined medium containing 0.5% sucrose, Alexa Fluor® 647-labeled dextran conjugate, and 0~10% CI, then cultured for 24 hours to form biofilm. Next, MT8148 organisms were stained with hexidium iodide and cultured for 24 hours, then 0~10% CI was added and incubation performed for three hours, and biofilm structures were observed using confocal laser scanning microscopy. Finally, to examine the antibacterial effects of CI, cultured MT8148 organisms were adjusted to OD_{600} = 0.1, then 0-20% CI was added and culturing performed at 37°C for six hours, followed by OD_{570} measurement.

Results: Cell amounts in biofilms were nearly the same with all CI concentrations used. Biofilms formed without CI showed abundant extracellular polysaccharides covering the surface as compared to those formed in its presence. Biofilm structures formed with added CI had lower levels of density and thickness as compared to those without CI. Antibacterial assay findings showed that OD_{570} was slightly decreased in a CI dose-

dependent manner.

Conclusion: These results suggest exfoliation activity by CI in *S. mutans*-related biofilm. Further studies are needed to clarify the detailed mechanism.

Streptococcus mutans Cell Surface Protein Expression Profiling in Non-alcoholic Steatohepatitis Patients

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Background: Approximately 20% of non-alcoholic fatty liver disease (NAFLD) patients are reported to progress from non-alcoholic fatty liver (NAFL) to non-alcoholic steatohepatitis (NASH). NASH has become one of the most common hepatic diseases in children. We previously reported that *Streptococcus mutans* organisms with both cell surface collagen-binding protein (Cnm) and 190-kDa cell surface protein antigen (PA) induced NASH aggravation. The present study was performed to analyze expression profiling of these proteins in *S. mutans* organisms isolated from NAFLD patients.

Methods: Using saliva specimens collected from 40 biopsy-proven NAFLD patients (NASH n=20, NAFL n=20), *S. mutans* organisms were isolated. PCR was performed to detect the *cnm* gene encoding Cnm using genomic DNA extracted from those organisms, while PA expression was confirmed by western blotting. Total RNA was extracted from *S. mutans* and complementary DNA synthesized by reverse transcription. Real-time reverse transcription PCR was used to evaluate expression levels of *cnm* and the *pac* gene encoding PA.

Results: The percentage of Cnm- and PA-positive (Cnm^+PA^+) strains in samples from the NASH patients was significantly greater than in those from NAFL patients ($P<0.05$). Although the expression level of *cnm* was not significantly different, that of *pac* was significantly higher in Cnm^+PA^+ *S. mutans* organisms isolated from NASH patients ($P<0.05$). Additionally, the expression level of *pac* in Cnm^+PA^+ *S. mutans* in NASH

patient samples was significantly higher as compared to that in Cnm-negative and PA-positive ($Cnm^{-}PA^{+}$) *S. mutans* organisms ($P<0.05$).

Conclusion: These findings indicate an association of $Cnm^{+}PA^{+}$ *S. mutans* organisms exhibiting a high level of *pac* expression with NASH aggravation.

Biofilm formation associated with oral bacterial dominance in pediatric patients affected by aggressive periodontitis

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Background: Early primary tooth loss is caused by rapid alveolar bone destruction and diagnosed as aggressive periodontitis. In our previous study, *Actinomyces* and *Veillonella* species were frequently detected in affected patients. *Actinomyces naeslundii*, an indigenous oral bacterium, produces peptidoglycan, which induces production of inflammatory cytokines and activates osteoclasts, leading to alveolar bone resorption. The present study investigated biofilm formation by *A. naeslundii* isolated from patients with rapid alveolar bone destruction.

Methods: Patients with aggressive periodontitis were divided into Group 1, which had a greater number of *Actinomyces* species as compared to *Veillonella* species detected, and Group 2, with a greater number of *Veillonella* species detected. A total of 82 *A. naeslundii* strains were isolated from Groups 1 and 2, and used in this study. Qquantitative analyses of biofilm formed by those strains were performed using Brain Heart Infusion (BHI) containing 1% sucrose.

Results: There was no significant difference between Groups 1 and 2 for biofilm quantity after 24 hours of incubation. In contrast, after 48 hours of incubation, that quantity was significantly greater in Group 2 ($P<0.05$).

Conclusion: These results indicate that the quantity of biofilm formed by *A. naeslundii*

from patients showing *Veillonella* species dominance was greater as compared to those in whom *Actinomyces* species were dominant. Further studies are needed to clarify the association of these two species in regard to biofilm formation and periodontitis manifestation.

Pathogenicity of collagen-binding protein in *Streptococcus mutans*

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Background: The most common form of chronic glomerulonephritis is IgA nephropathy (IgAN) and accounts for half of all pediatric cases. *Streptococcus mutans*, a Gram-positive, facultative anaerobic bacterium is known to be a major pathogen of dental caries. We previously reported that *S. mutans* with collagen-binding protein (Cnm) was frequently detected in the oral cavity of IgAN patients. The present study was conducted to investigate the pathogenicity of Cnm using silkworm and animal models infected with recombinant Cnm (rCnm).

Methods: The *cnm* gene, which encodes Cnm of *S. mutans* TW871, was ligated to pGEX6p-1, a protein expression vector. Using a mass culture of *E. coli* BL21, rCnm was obtained and transformed by the constructed plasmid. Several different concentrations of rCnm protein were injected through the dorsal surface of silkworms into the hemolymph, after which survival was determined every 12 hours for 204 hours. In addition, rCnm was into administered the jugular vein of specific pathogen-free Sprague-Dawley rats (4-week-old males), then the kidneys were evaluated following immunofluorescent staining with IgA antibodies.

Results: Among the different concentrations of rCnm administered to the silkworms, a significant difference for survival after 204 hours was noted between the group injected with PBS and that with rCnm at 50 μg. Furthermore, immunofluorescent staining of rat kidneys with IgA showed significantly greater IgA deposition in the mesangial region in the rCnm-treated as compared to the PBS-treated group.

Conclusions: These results suggest that Cnm is an *S. mutans* pathogenic factor related to occurrence of IgAN.