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Chapter 1 : Yixin Shi | iSearch

Porphyromonas gingivalis GAI was obtained from Dr E. Hoshino (Niigata University School of Dentistry), TDC60, TDC, and TDC were obtained from Dr K. Ishihara (Tokyo Dental College), and SU63 was obtained from Dr M. Yoneda (Fukuoka Dental College).

Download as PowerPoint Slide Figure 1: Black region of Arg-gingipain gene, signal sequence; dark region of Arg-gingipain gene, amino-terminal domain; open region of Arg-gingipain gene, proteinase domain; hatched region of Arg-gingipain gene, carboxyl-terminal domain. Restriction sites in parentheses no longer exist on the plasmids. Briefly, the culture of E. The cell pellet was resuspended in prewarmed enriched BHI broth and spotted on enriched tryptic soy agar. Erythromycin-resistant Em transconjugants were obtained at the frequency of 2. The cells were then harvested by centrifugation, washed with the electroporation solution mM sucrose , and resuspended in 0. Electroporation was performed on the condition voltage, 2. Tetracycline-resistant Tc transformants were obtained at the frequency of 3. The resulting supernatant was used as a culture supernatant in this study. On the other hand, bacterial cells were washed with phosphate-buffered saline PBS and resuspended in 10 mM sodium phosphate buffer pH 7. Gel Electrophoresis and Immunoblot Analysis Sodium dodecyl sulfate SDS -polyacrylamide gel electrophoresis was performed according to the method of Laemmli The proteinase inhibitor leupeptin was added to a solubilizing buffer to avoid proteolysis by endogenous proteinases. For immunoblotting, proteins on SDS gels were electrophoretically transferred to nitrocellulose membranes according to the method of Towbin et al. The blotted membranes were immunostained with anti-Arg-gingipain IgG, essentially according to the procedure described previously The casein- and hemoglobin-hydrolyzing activities were determined by measuring acid-soluble products by the method described earlier 42 with slight modification. The reaction mixture 0. The reaction was stopped by adding 0. The fluorescence was measured at nm excitation at nm on a fluorescence spectrophotometer model F; Hitachi, Tokyo, Japan. In summary, sterilized oyster glycogen 0. The cuvette that contained the reaction mixture consisting of 0. The intensity of light emitted in the cuvette was measured for 30 min. The CL response is expressed by the peak intensity of CL. Hemagglutination Assay Overnight cultures of P. The bacterial suspensions were then diluted in a two-fold series with PBS. The hemagglutination titer was determined as the last dilution exhibiting full agglutination. Since it was difficult to use this strain for construction of Arg-gingipain-deficient mutants because of its low efficiency in mobilization and electrotransformation, 2 we chose P. To examine whether the Arg-gingipain gene is located on the chromosome of P. One of the gene loci The rgpA locus was also hybridized with two oligonucleotide probes for the carboxyl-terminal domain of Arg-gingipain probe IV and for the region downstream from the Arg-gingipain gene probe V , whereas the rgpB locus was hybridized with neither of them Fig. The result suggests that the rgpA locus may encode the carboxyl-terminal domain of Arg-gingipain in addition to the proteinase domain, while the rgpB locus may not. Recently, Pavloff et al. Comparison between the amino acid sequences of Arg-gingipain gene of and Arg-gingipain-1 gene of H66 revealed that their proteinase domains were identical but their carboxyl-terminal domains were different Fig. A DNA region intervening between the first repeating sequence and the third one is completely deleted from the carboxyl-terminal region of the Arg-gingipain gene. To determine whether the rgpA locus of ATCC contains this intervening sequence, the oligonucleotide probe for the intervening sequence probe VI was used. From these Southern analyses, we also found that at least two separate chromosomal regions other than the rgpA locus might encode the carboxyl-terminal domain of Arg-gingipain

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Chapter 2 : Yixin Shi â€“ Scholarly Works â€” Arizona State University

Porphyromonas gingivalis is a gram-negative, non-motile, anaerobic bacterium implicated as a major pathogen in periodontal disease. P. gingivalis grows as black-pigmented colonies on blood agar, and many bacteriologists have shown interest in this property.

Abstract We have mapped a group of virulence genes of Porphyromonas gingivalis to a single large fragment of the genome. These genes rgpA, kgp, and hagA all contain a consensus repeat sequence HArep. Genomic DNA fragments separated by pulse-field gel electrophoresis were blotted and probed in order to localize the genes to a 0. Further hybridization analyses with single- and double-restriction digestion allowed us to generate a physical map of the fragment and determine the precise locations of the protease and hemagglutinin genes. In addition, we found an insertion-like sequence, IS, near the ends of the 0. A similarly sized fragment carrying HArep sequences was also demonstrated in the P. Porphyromonas gingivalis is an anaerobic, asaccharolytic bacterium that is recognized as an important etiologic agent in adult periodontitis 7 , 18 , Virulence factors of P. An allelic-exchange mutant of P. Similar results have been obtained with naturally occurring and allelic-exchange lysine-specific protease mutants of P. The specific role of such proteases in virulence has not been elucidated, but they might contribute to the ability of the bacteria to colonize the oral cavity by the exposure of cryptic sites and binding to an extracellular matrix, the evasion of host defense mechanisms through the hydrolysis of immunoglobulin and complement proteins, and the alteration of neutrophil antimicrobial activity by degradation of bactericidal proteins and acquisition of essential nutrients 1 , 14 , 15 , 17 , 24 , 32 , 35 , While the cysteine protease activity with arginine specificity originates from two different genes, rgpA and rgpB 24 , 31 , the lysine-specific cysteine protease activity is derived from a single gene, kgp formerly called prtP A nucleotide sequence of approximately 1. This sequence is known as the HArep consensus Both in vitro and in vivo studies have provided evidence that the protein sequence HArep encoded by this region might have a role in virulence properties. Another monoclonal antibody, 61BG1. In addition, the HArep contains the hemoglobin receptor domain Protoheme is an essential nutrient for P. Thus, it appears critical that P. Furthermore, the HArep consensus is flanked by bp of conserved repeated nucleotide sequence CRS encoding 46 amino acids Fig. This amino acid sequence contains motifs implicated in binding to fibronectin, collagen, and laminin Other studies have demonstrated 30 that arginine- and lysine-specific cysteine proteases have the ability to bind to fibrinogen, fibronectin, and laminin. We have used this information to locate and position HArep-containing genes on the W83 genome.

Chapter 3 : Porphyromonas gingivalis - microbewiki

P. gingivalis colonies grown on blood agar. Heme from the media is oxidized by the bacteria to produce hemin which accumulates on the cell surface producing a characteristic black pigment after about 7 days of anaerobic incubation.

Abstract Porphyromonas gingivalis, a gram-negative anaerobic bacterium associated with active lesions of chronic periodontitis, produces several proteinases which are presumably involved in host colonization, perturbation of the immune system, and tissue destruction. The aims of this study were to investigate the degradation of human transferrin by gingipain cysteine proteinases of P. Analysis by polyacrylamide gel electrophoresis and Western immunoblotting showed that preparations of Arg- and Lys-gingipains of P. Interestingly, gingival crevicular fluid samples from diseased periodontal sites but not samples from healthy periodontal sites contained fragments of transferrin. By using 55Fe-transferrin, it was found that degradation by P. Subsequent to the degradation of transferrin, bacterial cells assimilated intracellularly the radiolabeled iron. Our study indicates that P. Periodontitis is a chronic inflammatory disorder of the periodontium initiated by an overgrowth of specific bacterial species and characterized by the destruction of tooth-supporting connective tissues, including the alveolar bone. Porphyromonas gingivalis, a gram-negative anaerobic bacterium, has been implicated as a major etiological agent in the onset and progression of chronic periodontitis Arg- and Lys-gingipain cysteine proteinases, which occur in multiple molecular forms due to proteolytic processing of the initially translated polypeptides, are the main endopeptidases produced by P. Two genes code for Arg-gingipains rgpA and rgpB , and one gene codes for Lys-gingipain kgp 12 , 15 , An increasing number of reports have stressed the potential roles of Arg- and Lys-gingipains of P. The critical contribution of gingipains in the pathogenicity of P. The present study was aimed at investigating the degradation of human transferrin by P. The mutants were constructed by allelic replacement mutagenesis or integration of a suicide plasmid, as described previously 24 , Bacteria were grown anaerobically N2-CO2-H2, To maintain selective pressure and prevent the appearance of revertants, tetracycline 0. Before the mutants were used, their phenotypes were confirmed by testing their ability to cleave the chromogenic synthetic substrates for Arg-gingipain benzoyl-Arg-p-nitroanilide and Lys-gingipain N-p-tosyl-Gly-Pro-Lys-p-nitroanilide , as described previously 6. Use of these mutants facilitated purification of the gingipains, which are difficult to separate from one another. Arg- and Lys-gingipain activities were quantified by using benzoyl-Arg-p-nitroanilide and N-p-tosyl-Gly-Pro-Lys-p-nitroanilide, respectively 6. One unit of enzyme activity was defined as the amount of enzyme required to release 1 nmol of p-nitroaniline per h. Degradation of human transferrin by gingipains. Equal volumes of apotransferrin iron-free form or holotransferrin iron-saturated form 0. Undegraded transferrin and transferrin fragments were visualized following development in mM carbonate buffer pH 9. Autoradiography analysis for detection of 55Fe following degradation of transferrin. After this, the final concentration of transferrin was determined by the method of Lowry et al. Uptake of iron from transferrin by cells of P. Uptake of 55Fe from 55Fe-transferrin by P. The bacteria were grown to the late exponential growth phase in THB containing vitamin K but no hemin. In the absence of hemin, growth of P. This corresponded to a concentration of 2. The radioactivity associated with the bacteria was quantified with a gamma counter. Growth studies with P. Formation of hydroxyl radicals. In one control, holotransferrin was replaced by apotransferrin, the iron-free form, or by deionized distilled water. An additional control consisted of replacing the enzyme preparation by PBS. All buffers, as well as the gingipain preparation, were treated with Chelex Bio-Rad Laboratories Ltd. In addition, all glassware was washed with 6 N HCl and extensively rinsed in deionized distilled water. The final volume of all reaction mixtures was 0. The instrument settings were as follows: Two independent experiments were carried out to demonstrate the reproducibility of the results. Detection of transferrin fragments in gingival crevicular fluid. The pocket depth of each site was measured with a Michigan periodontal probe. Patients were distributed into four categories: The paper strips were then removed, and the samples were kept frozen until

they were used. Transferrin and transferrin fragments were visualized by Western immunoblotting, as described above. Apotransferrin appeared to be more susceptible than holotransferrin to degradation by either preparation of gingipains since no lower-molecular-mass fragments accumulated, suggesting that there was production of fragments too small to be detected by the immunological analysis used. This indicated that the presence of iron may stabilize the protein and partially protect it from degradation by gingipains. Although some fragments may have been similar, the degradation profile of holotransferrin produced by the Arg-gingipain A-Arg-gingipain B preparation differed to some extent from that produced by the preparation of Lys-gingipain. Several fragments with molecular masses between 40 and 80 kDa were produced. Incubating transferrin iron-free or iron-saturated form with both preparations of gingipains resulted in a profile comparable to that generated with the Lys-gingipain preparation. When human serum was used instead of commercial transferrin, degradation was also observed data not shown.