Thesis Tittle " The Study for the Purification of Outer Membrane Complex Antigen of Bacteroides fragilis and the Production of Specific Antibody "

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ABSTRACT

The outer membrane complex of Bacteroides fragilis ATCC 23745 was isolated with use of gentle techniques of heat, ethylenediaminetetraacetate (EDTA) treatment, shearing, and differential centrifugation. Relative purity of the preparation was suggested by the formation of a single band in a sucrose density gradient at a density of 1.21 g/ml. At this density was analysed as an outer membrane. The outer membrane complex component including capsular polysaccharide and protein was separated from outer membrane lipopolysaccharides (LPSs) by using column chromato-

graphy with Sephadex G-200 and used 0.5 per cent sodium deoxycholate buffer to disaggregate the LPSs. The major chemical compositions of the purified outer membrane consisted of protein (39 per cent), and carbohydrate (24 per cent). Analysis of the protein components by electrophoresis on polyacrylamide gel, the major proteins were found with the molecular weights of 26,000, 27,000, 39,000, 42,000, 45,000, 52,000, and 65,000 daltons. Immunodiffusion analysis of the purified outer membrane against the homologous whole cell antiserum gave one precipitin line.

Purified outer membrane complex preparation along with the whole viable B. fragilis cells were used to develope hyperimmune sera in rabbits. The purified outer membrane complex antiserum and the whole cell antiserum were compared on the basis of their reactivity and specificity with the uses of immunodiffusion technique, tube agglutination and enzyme-linked immunosorbent assay(ELISA).

The purified outer membrane complex antiserum gave rather low titre, 1:320 by the tube agglutination test, or 1:16,000 by ELISA test; whereas the whole cell antiserum gave the titre up to 1:1,280 by the agglutination test and 1:128,000 by ELISA test. In an agar gel diffusion test, precipitin lines were found only when tested with whole cell antiserum. The ELISA inhibition test described had

exquisite sensitivity and was capable of detecting as little as 0.01 μg of protein present in the antigen preparation.

Specificity to detect other B. fragilis strains were studied and the results showed that the purified outer membrane complex antiserum reacted with some B. fragilis tested strains whereas the whole cell antiserum reacted with all B. fragilis tested strains. These results were suggested that the purified outer membrane complex antigen is not the common antigen as described by the previous studies. Whereas the serum elicited by whole cell antigen which was represented of both LPSs and capsular polysaccharide showed more species-specific. It was suggested that the capsular polysaccharide and protein was rather type specific antigen and the whole cell antigen was rather species-specific antigen.

Cross-reaction to other species and genera were only observed with B. asaccharolyticus ATCC 25280 for both antisera when tested by ELISA inhibition but in rather high concentration of inhibitor. When the agglutination test was used, this cross-reaction was observed only for the whole cell antiserum. This result showed that they may share some antigenic determinant in their capsules or past infection with B. asaccharolyticus in rabbits may be existed.