CHAPTER IV

DISCUSSION

Type-1 fimbriae are proteinaceous appendages projecting from the surface of many gram-negative bacteria (32). The presence and abundance of type-1 fimbriate bacteria in a culture was estimated by the presence and strength of haemagglutinating activity (7). As shown in table 3, it was concluded that S.typhimurium F885 was the most heavily fimbriated strain and suitable for fimbrial preparation.

The presence of type-1 fimbriae on S.typhimurium F885 was confirmed by the effect of various monosaccharides on its haemagglutinating activity against guinea pig erythrocytes.

It was important to estimate the inhibitory potency of different carbohydrates on a single culture of known HP because the MIC of a carbohydrate is influenced by the HP of the culture and by the number of MHD used in the test. The results in table 4 show that D-mannose and methyl α-D-mannopyranoside strongly inhibited the agglutination of GPE The agglutination was somewhat sensitive to some extent, to D-glucose but not to D-galactose, D-altrose, L-rhamnose,

lactose or sucrose. The inhibition pattern is similar to that in haemagglutination by type-1 fimbriae from S. typhimurium LT2 (55).

Old (56) studied the carbohydrate inhibition of haemagglutination by type-1 fimbriated S.typhimurium cells and showed that the α-configuration at the C-1 position in the mannopyranoside molecule and unmodified hydroxyl group at C-2, C-3, C-4 and C-6 of the D-mannose molecule were required for maximum binding to the fimbrial protein. The result obtained with S.typhimurium F885 fimbriae in this study agree with these observations and so confirm that these haemagglutinins are type-1 fimbriae. In view of the sensitivity and specificity of the haemagglutinin, these type-1 fimbriae may be ascribed a mannose-recognizing lectin.

To confirm the role of the fimbriae in bacterial adhesion required fimbrial preparations that were free from other bacterial surface antigens. This is relatively difficult to achieve since the fimbriae, bearing extremely hydrophobic protein, and are easily contaminated by other bacterial surface antigens such as outer membrane proteins and lipopolysaccharide.

A number of purification procedures have been published, suggesting that fimbriae from different strains possess different physiochemical properties. The method of

Salit and Gotschlich (62), with repeated precipitations with either ammonium sulfate, give quantitative yields only with fimbriae that aggregate readily. With our F885 strain, this method and method of Knutton et al. (102) resulted in poor yields of fimbriae and significant contamination with other surface protein (table 5). The method of Dodd and Eisenstein (101) which modified and greatly simplified the procedure of Korhonen et al. (107), takes advantage of the stability of fimbriae in concentrations of urea that dis-aggregate flagella, resulted in high yields of fimbriae and negligible protein contamination.

When analyzed in SDS-PAGE, type-1 fimbrial preparation was found to be pure and to have a MW of 19K (Fig.6e) A minor contaminant of a MW 18K (Fig.6e), which became enriched during purification, could indicate that another fimbrial type is associated with the strain or that it is one of the multiple conformations in SDS solution recently postulated for the type-1 fimbriae (105). However, analysis of purified type-1 fimbriae by immunoelectro phoresis demonstrates that only one of the antigens present in the crude extract was present in the final preparation (Fig.8,9). The fimbriae also retain their native morphology (Fig.7) and biological activities (table 6).

In mice infected orally with S.typhimurium, the Peyer's patch tissue of the small intestine represents a primary focus of infection as Carter and Collins have

demonstrated (99). Hohmann et al. (100) and Srisart et al. (108) have shown that Salmonella strains able to protect against mouse typhoid, soon appeared in the Peyer's patches where they multipled over the course of several days, Salmonella unable to proliferate in the Peyer's patches did not provide such protection.

In our studies of mouse protection, the fimbriate strain F885 was capable of inducing protective immunity, whereas none of the animals immunized with the non-fimbriate strain F492, were protected (table 8). Strain F885 and F492 both express the E.coli O Ag 8 as their sole O Ag. Despite other differences in these two strains, such as motility, genetic and phenotypic back ground, their most marked difference in vivo is the ability of strain F885 to multiply and persist with the small intestinal Peyer's patches while strain F492 is quickly eliminated (Fig.10).

It seem most likely that the markedly different immunogenicity of strain F885 and strain F492 is determined by their respective abilities to colonize the small intestinal Peyer's patches. Hohmann et al. (100) have shown that these two strain are markedly different with the strain F885 priming for much increased serum and intestinal IgA Ab responses. In addition, Ag replication by strain F885 with in the Peyer's patches probably results in more efficiently immunization of the whole animal.

The difference between the vaccinated and control groups in the incidence of death and distribution of challenge organisms in Peyer's patches and spleen, indicate that vaccination of mice with purified fimbriae from strain F885 provides active protection against the challenge with the C5 strain.

Two lines of evidence suggest that antifimbrial Ab was responsible for the protective effects observed. First, the fimbriae used for immunization were pure by generally accepted criteria (101). Second, animals immunized against the type-1 fimbriae of strain F885 were afforded significant protection against challenge with strain C5, a strain that differed from F885 with regard to the O antigens but that shared other surface antigens.

These results agree with those reported by Srisart et al. (108), who have shown that there was no correlation between protection and the specificity of the O somatic antigens of the immunizing and challenge strains, the IgA antibodies measured were not responsible for protection, though probably an indication of it.

A likely explanation for the protection observed, is that it is due to antifimbrial antibodies which probably prevent adhesion of strain C5 to the epithelial cell surfaces. Presumably, Ab to fimbriae prevented adherence by blocking the mannose-specific binding domains of the

fimbriae.

Levine et al. (92) demonstrated that protection was mediated by a mechanism that was not bactericidal and probably involved Ab at the mucosal surface that interfered with adhesion of bacteria to critical mucosal sites.

Perry et al. (109) demonstrated that when fimbriate bacteria were incubated with antifimbriae Ab, they became clumped leading to better phagocytosis by leukocytes. However, the fact that adherence was also prevented by monovalent Fab' fragments, which bind to the fimbriae but were incapable of causing agglutination, indicated that the Ab had a direct effect on the adhesive properties of the bacteria (110).

Since S.typhi is exclusively a human pathogen, and remains a serious public health in Thailand and other developing countries (111,112). A suitable vaccine to control Salmonellosis is not currently available. At present, various typhoid vaccines are in use around the world, but all consist of whole cells killed in different ways, such as heat killed-phenol preserved, acetone killed. Although many of these parenteral vaccines have been shown in several controlled field trials to confer significant protection to persons living in areas where typhoid fever is endemic (113,114,115,116,117,118), they do frequently cause adverse reactions such as, fever, intense local

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2.2 as mannose - recognizing lectin, the α-configuration at the C-1 position and unmodified hydroxyl group at C-2, C-3, C-4, and C-6 of D-mannose molecule are required for maximum binding to the fimbrial protein.

2.3 the diameters are 6 nm and retain their native morphology in the purification process.

2.4 have a approximate MW of 19K.

2.5 as immunogen, capable of inducing protection in mice.

3. Fimbriate bacterial strains are able to confer protection against a virulent mouse typhoid strain where as non-fimbriate strains cannot.

4. The LPS of protective strain seem to plays no part in protection.

5. Type-1 fimbriae afford significant protection against challenge with mouse typhoid.

6. The data are consistent with the hypothesis that type-1 fimbriae of strain C5 act as virulence factors by facilitating adhesion to intestinal epithelia.