CHAPTER I.

INTRODUCTION

During the last ten years it has been shown that the adhesion of pathogenic bacteria to the epithelial cells of their hosts plays an important role in various infectious processes (1,2,3,4,5,6). Although at present only few adhesion mechanisms are evident at the molecular level, it has become apparent that bacterial adhesion to host epithelium is specific, i.e. pathogenic bacteria are able to bind selectively to the site of infection and that different adhesion mechanisms are functioning in different clinical situations. The advantage to bacteria of adhering to host epithelium is that it helps them to resist host defence mechanisms, such as voiding, by peristalsis, coughing, or urination and to colonize and invade tissues.

Enteric fever caused by some strains of Salmonella was one of the first infectious diseases in which the importance of adhesion was discovered. Duguid et al. (7, 8) suggested that the fimbrial adhesion of Salmonella to the gut epithelial cells might be marginally advantageous to the bacteria, either by facilitating the establishment of infection or by prolonging their carriage in the intestine. Subsequent studies have shown that such

bacterial adhesion, in most cases mediated by specific fimbriae, is a prominent feature in the pathogenecity not only of Salmonella (9,10) but indeed of all bacteria that infect epithelial surfaces, such as Escherichia coli (11), Klebsiella (12), Shigella (13),Neisseria gonorrhoeae (14), Pseudomonas aeruginosa (15), Gardnerella vaginalis (16) etc.

1. Bacterial Adherence

Many bacteria are known to be adhesive, attaching to and living in close association with various surfaces in their natural habitat. The ability of many pathogenic bacteria to adhere to specific host tissues is a factor of primary importance in diseases such as bacterial diarrhoea gonorrhoeae, and urinary tract infection (1,4,17,18,19). Specific adherence plays two important roles.

1. It allows the bacteria to resist and circumvent the flushing and cleaning mechanisms that protect many epithelial surfaces in higher animals.

2. It determines the site of microbial infection by facllitating specific surface-to-surface interaction between the bacteria and the host epithelium.

Adhesion of indigenous microorganisms to epithelial surfaces in higher animals plays an important role in the

maintenance of the normal flora of such ecosystems and indirectly protects the host by providing competition for incoming pathogens (20).

1.1 Specificity of the adherence of bacteria to mucosal surfaces

Interactions between bacteria and host represent the outcome of a complex series of events and involve not only non - specific types of binding such as electrostatic forces but also more specific binding. The specificty of the interaction of bacteria with host tissue was first suggested by *in vivo* evidence. Gibbons (21) has pointed out the apparent preference of particular bacteria for certain tissues over others (tissue tropism). For example, *Streptococcus pyogenes* is virtually limited to the nasopharynx skin and genito-urinary tract and rarely colonizes the nasopharyngeal cavity (22).

This concept of specificity by the interaction between bacteria and host tissue is further supported by the species specificity of certain bacterial infections Cheney et al. (23) demonstrated rather convincingly, the high degree of species specificty of a diarrhoeagenic strain of *E.coli* that was able to produce diarrhoea in rabbits but not guinea pigs and rats.

Perhaps even more convincing has been the evidence

that the susceptibility to certain infections is a genetic trait. Sellwood et al. (24) and Rutter et al. (25) have shown, for example, that certain pigs are genetically immune to E.coli K88 infections. By cross - breeding susceptible and resistant pigs, these researchers were able to show that susceptibility is coded for by autosomal dominant genes and moreover, is dependent on receptors for E.coli K88 on the brush borders of the intestinal epithelial cells.

1.2 Non-specefic factors influencing bacterial adherence

The mucosal surfaces of the healthy host are usually impervious to pathogenic organisms because of a number of cleansing mechanisms that operate on these surfaces. Mucosal surfaces, during good health, are constantly bathed by secretions laden with antibacterial enzymes and antibodies, which impede the attempts of pathogens to colonize the surfaces. The unattached organisms are then simply swept away in the luminal content, by mechanical means such as coughing, sneezing, ciliary action, swallowing, peristalsis, and excretion.

Pathogenic organisms are able to gain a foothold only by taking advantage of impaired local defense mechanisms. Even then, organisms that are able to attach may be eliminated by desquamation of the colonized

epithelial cells. Successful pathogens are those capable not only of penetrating the local defenses and attaching to mucosal cells, but also of replenishing the new surfaces as colonized cells are desquamated followed by invasion of epithelial cell barrier, either by the organism itself or by an excreted toxin.

Several non-specific factors are important determinants in the formation of multiple bonds between bacteria and host cells. The inherent net charge on the surface of both the bacterial and cells are of the same sign. These repulsive forces, however, may be overcome by the attractive forces between the hydrophobic molecules present in varying numbers on the two cell surfaces.

1.3 Specific binding

Once the nonspecific attractive forces overcome the electrostatic repulsive forces, the organism can approach the host cell surface and attach in a rather loose, reversible fashion. Permanant anchoring to the surface requires the formation, then, of many specific lock and key bonds between complementary molecules on each cell surface as is shown diagrammatically in figure 1. The complementary receptor and adhesin molecules must be accessible and arranged in such a fashion that many bonds can form over the area of contact with the cell surface.

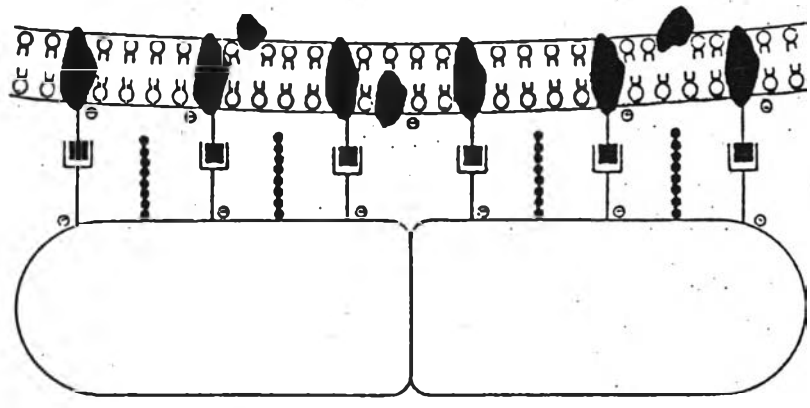

Fig. 1 Attachment of bacterial cells (bottom) via specific adhesins (fork-like structures) to complementary receptors (■) on the host cell membrane (top). To overcome the net negative charge (⊖) on both the bacterial and the host cell surfaces, hydrophobic molecules (regular black structures shown inserted in the host cell membrane) are attracted toward the hydrophobic phospholipid molecules (circles with legs) in the lipid bilayer membrane. The irregular black structures represent protein or glycoprotein into the host cell membrane.

The term adhesin, first coined by Duguid (12), is perferred to the adhesive structures on the surfaces of microorganisms. Receptors are complementary adhesive structures on the surfaces of host cells.

There is some evidence though indirect to show that specific receptor and adhesin molecules mediate the binding of a particular species of bacteria to host cells;

1. The adherence of the bacteria to tissue cells is inhibited by adhesin or receptor analogues, by enzymes and chemicals that specifically destroy adhesins and receptors and by antibodies to specific surface components (26,27,28).

2. The bacteria can bind to receptor analogues (28).

Direct evidence demands the isolation and purification of the bacterial adhesins and host cell receptors, and then showing that the isolated receptor binds to specific sites on the bacterial surface where as, the isolated adhesin binds to specific receptors on the surfaces of epithelial cells. The isolated materials, must of course, be able to block bacterial adherence, as is shown diagrammatically in figure 2. So far the degree of purification of adhesins and host cell receptors precludes such demonstration.

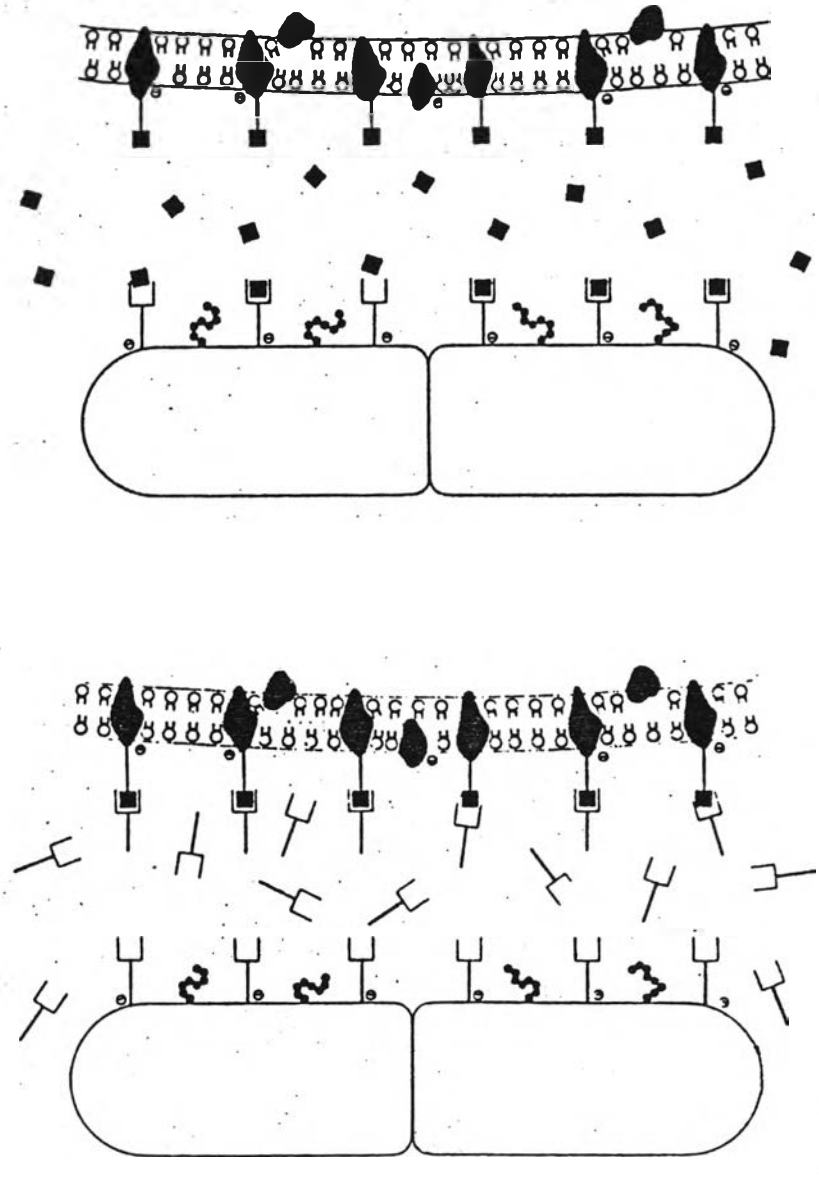

Fig. 2 Specific blockade of bacterial adherence by an excess of (top) isolated receptor analogue material (■) or (bottom) isolated or adhesin analogue material (fork-like structures)

2. Different Types of Fimbriae in Gram-Negative Bacilli

Bacterial fimbriae, or pili, are nonflagellar filamentous appendages on the bacterial surface (29,30,31) (The term fimbriae, meaning "fibers" or "threads" in Latin and was introduced by Duguid et al. (32). Brinton (33) later coined the name pili, meaning "hair" in Latin.) Since then, these two terms have generally been used as synonymus for the organelles responsible for the adhesive properties of bacteria, although Ottow (31) argued that the term fimbriae should be reserved for nonflagellar structures not involved in conjugation.

Since fimbriae were originally described in E.coli by Houwink and Van Iterson (30), several types of these filaments differing in morphology and adhesive properties have been observed in different species of gram - negative bacilli. For convenience, the classification of the fimbriae produced by these bacteria is that devised by Duguid et al.(7).

2.1 Type-1 fimbriae

These are relatively thick and they bear the mannose - sensitive haemagglutinin (MS adhesin) defined by Duguid (12). Up to 400 fimbriae are found per cell in a peritrichous arrangement. They average 70 Å in diameter and 2 um in length (34). Type-1 fimbriae are present in

most fimbriate strain of E.coli (32), Enterobacter cloacae (12), Shigella flexneri (13), Klebsiella aerogenes and Serratia marcescens (12) and many serotypes of Salmonella (7).

2.2 Type-2 fimbriae

Type-2 fimbriae produced by some Salmonellae (6, 35) are the non - haemagglutinating fimbriae and devoid of adhesive affinity for animal, plant and fungal cells. Morphologically indistinguishable from type-1 fimbriae, they are sufficiently related antigenically for Old and Payne (36) to suggest that type-2 fimbriae are non-adhesive forms of type-1 fimbriae.

2.3 Type-3 fimbriae

These are the "thin - type" fimbriae, 48 Å in diameter bearing a mannose - resistant haemagglutinin (MR adhesin) found by Duguid (12) in almost all strains of K. aerogenes and Ser.marcescens. Most of these strains also possessed type-1 fimbriae. They mediate bacterial adhesin to fungal cells, plant cells, cellulose fibers and glass, but not to animal cells unless these are fist modified by chemical or physical means (12). The unique substrate affinity of type-3 fimbriae led Duguid (12,37) to suggest they may confer some advantage on bacteria that grow as saprophytes in the soil.

2.4 Type-4 fimbriae

These are the fimbriae of Proteus sp. (35,38,39, 40). They are very thin, about 40 Å in diameter and bear a mannose-resistant (MRP) haemagglutinin that differs from the MR haemagglutinin of type-3 fimbriae in being active on fresh, untanned red cells.

2.5 Type-5 fimbriae

These are the monopolar fimbriae of Pseudomonas echinoides. They are about 48 Å in diameter and bear a mannose-sensitive adhesin. The fimbriae bacilli adhere by their poles to sheep red blood cells but, because of the monopolar distribution of the fimbriae, they do not bind the cells together and cannot produce haemagglutination (7).

2.6 Type-6 fimbriae

These are very long (up to 10 µm), thick (96 Å in diameter) and scanty (4-40 per bacillus), and either are non-haemagglutinating, in K.ozaenae (12).

2.7 Other fimbriae

MR adhesins have now been found in many strains of enterotoxigenic E.coli (ETEC) from different sources.

These fimbria can be distinguished by their serological and haemagglutination properties, but a property common to them all was found to be plasmid mediated and perferentially expressed after growth on solid medium at 37°C but not after growth at 18°C or in broth (41).

K88 (42,43) and 987P antigens (44,45) are fimbrial proteins which occur on E.coli strains causing diarrhoea in piglets. ETEC strains from calves and lambs carry fimbria termed K99 (46,47) and F41 antigens (48). Similar colonization factors were subsequently described in strains of ETEC causing diarrhoea in man: CFAI (49) and CFAII (50) by Evans et al., and E8775 (51) by Rowe et al. E.coli strains causing pyelonephritis in humans carry P-fimbriae, which bind to P-blood-group-specific glycosphingolipids on human urinary tract epithelial cells (52,53).

3. Fimbrial Adhesins of Salmonella

3.1 Characterization of type-1 fimbriae

Most Salmonella species are able to grow type-1 fimbriae (7), and their synthesis has been shown to be chromosomally coded in S.typhimurium (54).

type - 1 fimbriae of Salmonellae are protein filaments that are similar in morphology to those of E.coli and Sh.flexneri. Richly fimbriated cells bore 200-300

fimbriae mainly between 0.2 and 1.5 μ in length and a little less than 10 μm in width (7). Korhonen et al.(55) have demonstrated that type-1 fimbriae in purified form obtained from S.typhimurium are chemically different from those of E.coli.

Type-1 fimbriae of S.typhimurium LT2 consist of one protein subunit with an apparent molecular weight of 21,000 hydrophobic amino acids comprise 40.3% of the total amino acid composition and the isoelectric point of the fimbriae has been reported to be 4.1 (55).

The fimbriate bacteria consequently have a lower density of negative surface charge and a more hydrophobic character than the non-fimbriate bacteria, and these properties will facillitate their close approach to the negatively charged, hydrophilic surface of erythrocytes and other cells to allow adherence at the hydrophobic fimbriae tips (34,17).

3.2 Haemagglutinating specificity and mannose sensitivity

All strains of type-1 fimbriate bacteria have the same pattern of haemagglutinating specificity for the red cells of most species e.g. guinea-pig, fowl, horse, mouse, pig, and rhesus monkey, very strongy, human cells of all blood groups moderately strongly, sheep and goat cells

weakly and ox cells not at all (17). The haemagglutinating activity is best developed in stationary-phase cultures grown serially for long periods at 37°C in static aerobic liquid media, and this phenotype is changed to a non-fimbriate and non-haemagglutinating phase when cultured serially on agar plates (7,13,32). During these changes, the strength of the haemagglutinating activity varied directly with the proportion of fimbriate bacteria in the culture.

After Duguid et al. (32) had observed that D-mannose, alone of many sugars, strongly inhibited an E.coli haemagglutinin, it was shown that similarly haemagglutinating activity of Sh.flexneri, Klebsiellae and Salmonellae was inhibited by small concentrations e.g. 0.01 - 0.5% (w/v) of D-mannose, methyl ∝-D-mannoside and yeast mannan (7,12,13) and a very few substances with molecular structures related to D-mannose (56). The mannose - sensitive haemagglutinin in such strains was designated the 'MS adhesin' by Duguid (12).

3.3 Adhesion to cells other than erythrocytes

Bacteria with MS adhesin, and no other adhesive factor, adhere strongly to many kinds of cells besides red blood cells. Duguid et al. (7) showed that type-1 fimbriate bacteria have a similar mannose - sensitive adhesiveness for ox, human and guinea-pig leucocytes; ox,

human, mouse and guinea-pig intestinal epithelium; cells of guinea-pig liver, kidney and tracheal epithelium; the protozoon Trichomonas vaginalis; the yeast cells of Candida albicans, the hyphae of Aspergillus niger and some other moulds; and the root hairs of red clover and cress seedling plants that had germinated under bacteria-free conditions and that the adhesion was inhibited by the addition of D-mannose. The ability of the bacteria to adhere to ox leucocytes and epithelial cells, though they could not adhere to ox erythrocytes, showed that an adhesin's affinity for erythrocytes does not necessarily correspond with its affinity for other kinds of cells from the same species of animal.

3.4 Phase variation and condition of culture

The formation of type-1 fimbriae may be subject both to spontaneous variation and to variation determined by the conditions of culture. Brinton et al. (57) were the first to show that the bacteria of E.coli strain B varied reversibly between a fimbriate and a non-fimbriate phase, the change appeared to be spontaneous and took place in either direction at the rate of about one per 1,000 bacteria, per generation. The predominant fimbrial phase in culture of Salmonellae was later found to be determined by the conditions; e.g. 48 h culture in static tubes of broth gave 50 - 95% fimbriate bacteria, whilst a few serial cultivations on agar plates produced cultures

with few or no fimbriate bacteria.

Culture in static broth favours the growth of fimbriate bacteria, because the fimbriae enable the bacteria to rapidly establish themselves in a thin pellicle on the surface of the broth, where the supply of atmospheric oxygen allows them to multiply to from a population several times greater than that of non-fimbriate bacteria in a culture without a pellicle (7,13,57,58,59). Some non-fimbriated bacteria, e.g. Sh.sonnei, K.edwardsii and some mutant strains of S.typhimurium, (Fim-, Inl-, Rha-) which are unable to ferment inositol or L-rhamnose in peptone water and do not give rise spontaneously to Fim+ mutants, can form non-fimbrial pellicles on static liquid cultures, but do so only at a later stage of growth (e.g. after 24-48 h) than fimbriate bacteria (6-10 h).

The variation of the fimbrial phase in bacteria cells may be subject to direct induction by particular environmental condition, for example the synthesis of type -1 fimbriae is dependent on cyclic AMP and is subject to catabolite repression by many carbohydrates (60). Furthermore, it has been shown that the phase variation of type-1 fimbriae is under transcriptional control (61).

3.5 Inhibition by D-mannose and its analogues

The powerful inhibitory action of D-mannose on adhesion by type-1 fimbriate bacteria extends to their

reactions with all types of animal, plant and fungal cells tested as substrates (7,13,62,63). Old (56) studied the carbohydrate inhibition of haemagglutination by fimbriated S.typhimurium cells. From among the many substances tested only D-mannose, methyl α-D-mannoside, 1,5-anhydromannitol, D-mannoheptulose and α-D-mannose 1-phosphate were strongly inhibitory; D-fructose was moderately inhibitory and 2-deoxy-D-glucose, 6-deoxy-D-mannose and methyl-β-D-mannopyranosides were weakly inhibitory, and showed that the -configuration at the C-1 position in the mannopyrano side molecule and unmodified hydroxyl groups at C-2, C-3, C-4 and C-6 of the D-mannose molecule are required for maximum binding to the fimbrial protein. The high specificity of the inhibitory action suggests that the sites with which D-mannose reacts are located on the bacterial fimbriae and not on the very diverse types of cells that serve as substrates for the fimbriae. Evidence that D-mannose reacts specifically with type-1 fimbriae of S.typhimurium in the absence of other cells was obtained in the finding that the early, fimbriae-dependent fomation of a surface pellicle on broth cultures was inhibited by the addition of D-mannose or methyl α-D-mannoside, but not by that of other fermentable or non - fermentable sugars (57,58).

Duguid and Gillies (13) found that the binding of D-mannose to its receptor sites was rapidly reversible, when either the bacteria or the red cells were suspended

in 2% (w/v) D-mannose solution in saline, they remained normally active in producing haemagglutination when the sugar was diluted out.

The simplest explanation of the inhibitory action of D-manose is that it serves as a soluble analogue of fixed D-mannose - like residues on the surface of erythro cytes and other cells, and so binds to and blocks adhesive sites on the fimbriae that otherwise would bind to the residues on the cells (28,64). If this is so, D-mannose-like residues must be present on the very wide range of animal, plant and fungal cells that serve as substrates for the MS adhesin. Other possible explanations of the action of D-mannose (17) are

1. that it binds to and covers hydrophobic groups on the fimbriae, making the fimbriae more hydrophilic and thus repellent to other cells, and

2. that fimbriae are allosteric proteins which the binding of D-mannose alters from a hydrophobic and adhesive to a hydrophilic and non-adhesive form.

3.6 MS adhesive sites on fimbriae

The relatively low electro - negative charge on bacteria with type-1 fimbriae and the relatively hydrophobic character of the fimbrial protien (65) is likely to facilitate a close approach of the bacteria to

cellular substrates, but by themselves these properties seem insufficient to account for the firm adhesion of the bacteria to the cells. The adhesion is more probably due to the presence on the fimbriae of specific sites that bind to receptor substances, perhaps D-mannose-like, on the substrates. The location and structure of such fimbrial adhesive sites remains to be determined.

The MS-adhesive fimbriae in different entero bacteria are not identical in composition, in as much as that is manifest by their antigenic character. Although there is a partial sharing of type-1 fimbrial antigens among the species E.coli, Sh.flexneri and K.aerogenes, and a partial sharing among the genera, Salmonella, Arizona and Citrobacter, there is no sharing or similarity of type -1 fimbrial antigens between these two groups of organisms (66,67).

Duguid and Old (17) have suggested that the MS adhesive sites occupy only a very small part of the surface of the fimbriae, their likeliest location would seem to be at the peripheral tips. In a watery medium, the fimbriae presumably radiate peritrichously from the bacteria owing to a mutual repulsion of their lateral surfaces, which must be sufficiently hydrophilic to prevent them binding together. The outward facing tips, which may be hydrophobic and bear the adhesive sites, are the parts of the fimbriae that will first come into contact with the

surfaces of cells and other substrates.

3.7 Function of the MS adhesin

The MS adhesin of parasitic enterobacteria may help them to colonize the intestinal, urinary or biliary tract by attaching them to the mural epithelium and so enabling them to resist removal by the outflowing luminal contents. It may also increase their opportunity to invade the epithelium. Most Salmonellae which are able to infect mammals form type-1 fimbriae; this suggests that the MS adhesive property confers at least a marginal advantage on these parasites (7). Some support for this view has been obtained in experimental oral infections of mice with genotypically fimbriate and non - fimbriate lines of S.typhimurium, in which the former line gave more numerous infections and longer periods of faecal dissemination than the later (8).

A further possible role of type-1 fimbriae is in the promotion of the conjugational transfer of DNA by non-specifically enhancing, and so stabilizing, the mutual adhesion of mating pairs of bacteria. Although DNA transfer is primarily dependent on the presence of sex pili in the donor bacterium, experiments on the transfer of the coli factor have shown that the rate of transfer between the donor and recipient strains is greatly increased if at least one of the strains forms type-1

fimbriae (68).

If the adhesive property of type-1 fimbriae has a useful function, its exercise may be advantageous only in some circumstances of the life of the bacteria. In other circumstances, the bacteria may benefit from being able to disperse from exhausted to fresh substrates. The value of being able to vary between an adhesive and a dispersive phase may be the reason why most bacteria that form type-1 fimbriae are capable of undergoing fimbria phase variation (12).

4. Prevention of Bacterial Adherence

Because of the likely importance of attachment in the pathogenesis of infectious diseases, considerable attention is now focused on the development of measures to prevent the adhesion of harmful bacteria to mucosal surfaces before the organisms can produce tissus damage.

4.1 Application of receptor analogues

Aronson et al.(69) studied the influence of intra vesicular instillation of methyl α-D-mannopyranoside (αMM) a competitor inhibitor of the binding of mannose by E.coli was tested for its ability to prevent infection of the urinary tract of mice with infective strains of the organisms. Injection of the bacteria in the presence of

the ∝MM resulted in a considerable reduction in the number of bacteriuric mice. Administration of methyl ∝-D-gluco pyranoside to another group of mice did not alter colonization as compared with that in control mice. The result of this study showed that a receptor analogue interferes with microbial adherence and colonization in vivo.

4.2 Sublethal doses of antibiotics

Recent studies (26,70,71,72,73) have indicated that sublethal dose of antibiotics may alter the ability of certain bacteria to adhere to epithelial cells. Eisenstein et al. (26) found that streptomycin did not suppress the formation of fimbriae but produce an aberrant fimbrial protein, possibly by causing misreading of messenger RNA, and these fimbriae, when isolated from the drug - grown bacteria, lacked the ability to agglutinate guinea pig erythrocytes.

4.3 Antiadherence vaccine

The use of lipopolysaccharides and capsules as vaccines has been hampered by the wide serological hetero geneity and the low immunogenicity of these antigens. Outer membrane proteins, which share serological cross-reactivity in enterobacterial species (74), may be hidden by capsular antigens and thus inaccessible to serum

antibodies. These problems may largely be overcome by the use of bacterial adhesins as vaccines.

The ideal candidate for a vaccine against bacterial adherence would be the isolated and purified adhesin itself. Other candidates would include other surface components that are able to evoke antibodies that bind to the surface of the pathogens and sterically block the adhesin from interacting with its receptors on host cells. To be effective, the blocking antibodies must, of course, reach the mucosal surfaces either actively immunity in the adults or passively in the offspring via maternal antibody from colostrum or milk. Thus, antiadhesive vaccines must be able to induce local immunity at accessible mucosal surfaces (75,76,77).

5. Fimbriae as Vaccines

With the realization that adhesion is a prerequisite for several bacterial infections, much interest has been focussed on the use of adhesion-mediating structures (adhesins) as vaccines (78,79,80). A number of different fimbriae have been tested as vaccines (table 1).

5.1 In vitro studies

Isaacson et al. (81) have shown that purified E.coli 987, K99 or type-1 somatic fimbriae adhered to

Table 1 Purified bacterial fimbriae used as vaccines

With fimbria antigen	Immunization in	Protection against
K88	Piglet	Diarrhoea
K99	Piglet,calf	Diarrhoea
987	Piglet	Diarrhoea
P-fimbriae	Monkey	Ascending pyelonephritis
Type-1	Rat	Ascending pyelonephritis
Gonococcal	Human	Gonorrhoea
Type-1	Human	Diarrhoea

epithelial cells *in vitro*, as did bacteria possessing these antigens. Purified pili competitively inhibited the attachment of *E.coli* bearing the homologous fimbrial antibody prevented attachment to epithelial cells of purified fimbriae or of *E.coli* bearing the homologous fimbrial antigen (81).

5.2 Veterinary studies

The capacity of purified fimbriae from various strains of *E.coli* to induce protective immunity against diarrhoea in domestic animals is well established.

The first fimbria that was used as a vaccine was the K88 antigen by Rutter and Jones (82). Because coli bacillosis in piglets occurs in the neonatal period ETEC disease is controlled by parenterally active immunization pregnent gilts to stimulate colostral antibodies which passively protect piglets suckled by successfully immunized mothers (82,83,84).

There have been several reports of similar experiments, in which purified or partially purified preparations of the K99 antigen have protected piglets and calves against experimental diarrhoea (83,85,86,87,88). In all such instances, high titres of K99 antibodies were demonstrated in the colostrum.

The first commercial vaccines produced via

recombinant DNA technology were based on K88 and K99 fimbriae (89).

Purified type-1 fimbriae of E.coli have been tested as a vaccine against ascending pyelonephritis in rats (90). Rats immunized with the fimbriae were significantly less infected and had lower contents of the challenge bacteria in their kidneys than did the control rats.

Currently the P-fimbriae of human pyelonephritogenic E.coli strains are being tested for protection against experimental pyelonephritis (91). Preliminary vaccination trials in monkeys have shown that immunization with P-fimbriae offers protection against provoked pyelonephritis by a heterologous P-fimbriated strain. The protection was correlated with anti-fimbrial antibodies in the urine of vaccinated monkeys.

5.3 Human studies

Because the veterinary sector has obtained extremely promising results in the prevention of neonatal diarrhoea in herd animals by the use of purified fimbriae as vaccines, Levine et al. (92) demonstrated that parental type-1 fimbrial vaccines in humans can stimulate antibodies without adversely affecting the intestinal functions or the normal E.coli flora. Protection against

experimental diarrhoea by a strain having both type-1 and CFAI fimbriae was seen only with the highest doses of vaccine (about 1.8 mg per person). However, due to contaminants in the vaccine preparation, immunization with fimbriae also led to production of anti-O antibodies which may have had a role in protection.

Several laboratories are currently developing fimbrial vaccines against gonorrhoea. Purified gonococcal fimbriae have been used to immunize humans (93). Adverse reactions were limited to reactions at the injection site and consisted of pain, erythema and induration. Immunization led to antifimbrial antibodies in the sera and to enhanced phagocytosis of the homologous strains.

6. Research Aims

Many investigators have used mice infected with the natural murine pathogen, S.typhimurium as a model for testing the efficacy of various vaccine preparations against mouse-typhoid (94,95,96,97,98).

The course of an infection with S.typhimurium in mice is well established (99,100). Following oral administration of a virulent strain to mice, the bacteria first adhere to the epithelial cells and subsequently colonize the mucosal tissues. They then invade the Peyer's patches of the small intestine, where they multiply within

reticuloendothelial cells, eventually producing infective foci, in both the liver and spleen. An overwhelming infection results, with very large numbers of bacteria present in the blood at the time of death of the infected animals.

Although the factors responsible for the virulence of Salmonellae have not been well defined, there is some evidence that fimbriae on the bacterial surface promote their adhesion and subsequent colonization. This study is an attempt to correlate the fimbriation of Salmonellae with their ability to produce enteric infection.

The research aims will be

1. To prepare purified fimbriae from S.typhimurium F885.

2. To study the properties of the type-1 fimbriae from S.typhimurium F885.

3. To compare the ability of fimbriate and non-fimbriate but otherwise similar strains to confer protection in mice against a virulent mouse typhoid strain.

4. to assess the protective value of a fimbrial preparation vaccine from S.typhimurium F885.