Mechanisms of Colonisation and Colonisation Resistance of the Digestive Tract

Part 1: Bacteria/host Interactions

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The digestive microflora is characterized by a high degree of complexity and diversity. Variation in the composition of the digestive flora of an individual occurs in space and time. Two kinds of strains can be defined: transient strains in opposition to resident strains, which are repeatedly isolated from the digestive tract over a long period of time. The latter can be assumed to have colonized the digestive tract. We will present here studies on the properties of bacteria implicated in the colonisation process. Firstly, we summarize data on the role of adherence in this process, the mechanisms involved in adhesion to mucosal structures (cellular and mucus glycoproteins, extracellular matrix components) and give examples of adhesion in various intestinal bacterial groups (commensal and potentially pathogenic bacteria). Secondly, we describe the role of glucidolytic and proteolytic enzymes involved in host-bacteria interactions and implicated in colonisation and maintenance of the indigenous intestinal flora. *Key words*: colonisation, colonisation resistance, bacterial adherence, bacterial metabolic enzymes.

INTRODUCTION

The digestive microflora is characterized by a high degree of complexity. Although adult individuals tend to have their own predominant species combinations that fluctuate little over time, there is a constant turnover of individual bacterial strains in the microflora. Unfortunately, studies on variations in the microflora at the strain level have only been carried out for very few bacterial groups. Only *E. coli* has today been systematically studied concerning the longitudinal colonisation patterns of individual strains, but the same pattern has been described for other enterobacteria, such as *Klebsiella* and *Enterobacter* (1). Tannock reported that certain ribotypes of bifidobacteria could be recovered repeatedly from a person's intestinal microflora while others appeared only transiently (2).

It is very difficult to deliberately implant new strains into the intestinal microflora of an individual. However, renewed colonisation occurs all the time, since there is a constant appearance of new bacterial strains in the microflora of an individual while others disappear. The temporal variation in the microflora of an individual was first studied by Sears 50 years ago. He noted that an individual typically harboured a few *E. coli* strains in the colonic microflora at one point in time. Some of these strains persisted for extended periods of time (months or years) in that individual. In contrast, other *E. coli* strains identified in the same individual would vanish from the microflora between two sampling occasions (3–5). He called the first type of strain 'resident' and the second type 'transient', terms that have been used ever since. Usually, a resident strain has to be repeatedly isolated over a period of at least three weeks in order to be defined as such. Despite the fact that many individuals in Western societies have prolonged intestinal transient times-even latex particles that are ingested may be excreted during one to two weeks—a strain repeatedly isolated from the faeces for more than three weeks has to be able to replicate in the intestine. Such a strain can thus be assumed to have colonized the individual.

The ability of a bacterial strain to colonize and persist in the microflora may be influenced by a number of factors. The first strain may have an advantage, as it can establish without having to compete with other strains for nutrients, growth factors, binding sites etc. Thus, it is relatively easy to experimentally colonize newborn infants with *E. coli* during the first days after birth. Subsequently a 'barrier' towards implantation of new strains successively develops

(6, 7). This 'barrier' is probably formed mostly by competition from other strains that are already established in the microflora, and the barrier effect is called 'colonisation resistance'. In addition, the ability to adhere to intestinal cells or mucus structures, as well as the ability to use certain substrates (which requires possession of certain enzymes) can influence the ability to colonize and persist in the gastrointestinal tract.

BACTERIAL ADHERENCE IN THE ESTABLISHMENT OF THE NORMAL INTESTINAL MICROFLORA

It is intuitively imagined and repeatedly stated that bacterial adherence to mucosal structures is a prerequisite for, or at least strongly facilitates, colonisation and persistence of individual bacterial strains in the normal intestinal microflora. Bacterial attachment allows bacteria not to be swept away by intestinal motility and secretion and to install in an appropriate ecological niche favourable for growth since nutrients tend to concentrate at solid-liquid surfaces. The *in vitro* capacity to adhere to any mucosal structures has even been suggested as a 'selection criterion' to identify probiotic strains which might be successful to colonize human beings or animals (8, 9).

However, the simple notion of adherence as a prerequisite for colonisation and/or persistence has by no means been proven. Few studies have directly addressed the issue and existing studies are limited to *E. coli*. This species carries a number of well-characterized adhesins, many of which confer adherence to mucosal structures. The lesson that can be learnt from *E. coli* is that adherence via certain specific adhesins to their reciprocal receptor structures in the intestine confers colonising capacity, whereas binding via other adhesin/receptor pairs apparently does not. Thus, adherence *per se* is no good predictor of colonising capacity. For intestinal bacteria other than *E. coli*, information on adhesins and their capacity to bind to gastrointestinal mucosa is at the best scanty. In fact, with the exception of one study of the adherence of a large number of human mucosal *Lactobacillus* isolates to a colonic cell line (10), only a few strains at a time have been examined for their adherence capacity. We will here summarize studies of the capacity of various intestinal bacterial groups to adhere to mucosal structures mainly in the human intestine and present data on the role of adherence in intestinal colonisation.

Role of bacterial adherence

The proximal small intestine contains only approximately 10^2–10^3 bacteria per ml of intestinal fluid. This paucity of microbes seems primarily to be caused by the forceful peristalsis in the small intestine (11). Since the flow of intestinal contents at this site exceeds the maximum rate of bacterial multiplication, only bacteria, which adhere to the mucosa, can persist (12). For pathogenic *E. coli* strains that colonize and infect the small intestine, adherence to the epithelium is therefore a prerequisite for colonisation as well as for the pathogenic effect (13).

In the colon, peristalsis is much more sluggish than in the small intestine. The bacterial population reaches immense numbers (approximately 10^{11}–10^{12} per gram of faecal contents) and only a small fraction of the bacterial mass is in close contact with the epithelium. The role of adherence for large intestinal colonisation may, thus, seem minor. However, adherence may place bacteria in a favourable position vis-à-vis their growth substrate. Colonic lumenal contents are a very poor growth substrate for bacteria (14, 15). Fresh mucin from mucosal goblet cells can be degraded by certain members of the microflora (16) generating mono- and oligosaccharides that in turn may be utilized as growth substrates by other bacteria (16). Further, the mucin is admixed with secreted and transudated glycoproteins and sloughed epithelial cells. The result is a complex blend termed mucus, which is an excellent substrate for bacterial growth (14, 15). Adherent bacteria may also get access to micronutrients that leak out of the epithelial cells (17). Lastly, the oxygen tension is higher close to the mucosa. This would favour the growth of facultative anaerobes, which grow more rapidly in the presence than in the absence of O_2. It can, thus, be anticipated that bacterial factors which promote colonisation of the mucus layer will enhance metabolism and thereby multiplication and persistence. Although it has been repeatedly shown that the species composition of bacteria cultured from faecal samples resembles that of intestinal biopsies (18, 19), the mucus layer probably contains most of those bacteria that are actively replicating. Indeed, it has been calculated that the generation time of *E. coli* in the mucus layer is between 40 and 80 minutes, whereas the luminal population is static (20).

Bacterial adherence mechanisms

Bacteria can interact with epithelial cells, with the extracellular matrix and the mucus layer. The mucus layer could represent a physical barrier and a competitive inhibitor to epithelial cell adherence but binding to mucus could also serve as a first step in the colonisation process (21). When the epithelium is intact, only the brush border membrane is available as adhesion sites, but if the mucosa is damaged, subepithelial structures may be uncovered to which bacteria might bind.

Autoaggregation of bacteria probably occurs via the binding of bacterial adhesins to their corresponding carbohydrate receptor, located in the cell envelope. This type of association may also occur between members of different bacterial species; a coaggregation between vaginal lactobacilli and *E. coli* was noted by Reid (22). The aggregated state may be favourable for intestinal bacteria, as has been observed with bacteria in other ecosystems (23).

Bacteria adhere to host target structures by means of adhesins, which are proteins recognising a defined carbohydrate sequence present on host cell glycoproteins or glycolipids, or less often, a defined protein structure. In addition, non-specific adherence mechanisms may be involved, including electrostatic forces and lipophilic/hydrophobic interactions. To demonstrate the lectin-carbohydrate nature of bacterial adhesion, inhibition of binding reactions by soluble saccharide structures may be utilized. In some instances, lectins from other sources than bacteria (e.g. plant lectins) that mimic the binding of bacteria to target cells have been used to infer the binding structure. The first demonstration of an adhesin specificity was in 1955, when Collier and Miranda showed that the haemagglutination by *E. coli*, which had been observed since the beginning of the century, could be inhibited by the monosaccharide D-mannose.

In Gram-negative bacteria, the adhesins are located on surface structures termed fimbriae, fibrillae or curli (24, 25). In Gram-positive bacteria, adhesins are usually located in the cell wall or surface coat (so-called afimbrial adhesins), although fimbrial structures have been demonstrated on, e.g., vaginal lactobacilli (26). A bacterium may carry one or several different adhesins. The synthesis of fimbriae and adhesins in Gram-negative bacteria may be switched on and off, depending on environmental conditions, a process called phase variation (25). Bacteria, which cannot regulate their adhesin expression, are inefficient colonizers (27).

Adherence to cellular and mucus glycoproteins

Mucosal surfaces are characterized by an extensive carbohydrate coat and the bacterial lectin-like adhesins use cellular glycoproteins, glycolipids or the associated mucin glycoprotein as receptors. Several adhesins of commensal as well as pathogenic bacteria can be classified as lectins. Lectins are proteins or glycoproteins, which exhibit a specific carbohydrate binding activity (28–30). Certain carbohydrate-specific bacterial adhesins may recognize the carbohydrate receptor both in an internal and terminal position. However, surrounding chemical groups could enhance or inhibit the binding (31). Both glycolipids and glycoproteins can serve as targets for bacterial lectins. The glycoproteins of the epithelial cell membrane are mostly of the *N*-linked variety (32), whereas those of mucin molecules are mainly *O*-linked (33).

The presence of bacterial adhesins can be demonstrated by binding of the bacteria to target cells, e.g., freshly isolated intestinal epithelial cells, or to cell lines of intestinal origin. These often derive from colonic adenocarcinomas, e.g., the cell lines HT-29, Caco-2 and Intestine 407, and more or less faithfully mimic the assortment of carbohydrate and other receptors found on mature intestinal epithelial cells, which are difficult to culture *in vitro* (34). However, malignant transformation may change the glycoconjugate processing of the cell and, hence, alter the repertoire of receptor structures on the cell surface (35). Receptor structures identified on epithelial cell lines should preferentially be identified also on freshly isolated intestinal epithelial cells. Cell lines of non-intestinal origin have also been used in adherence studies of intestinal bacteria, e.g., the pharyngeal cell line HEp-2, extensively used as target for binding studies of enteropathogenic *E. coli*, and the cervical cell line HeLa.

The carbohydrate sequences that are recognized by bacteria on intestinal epithelial cells may also be present on erythrocytes from the same or another animal species. This phenomenon conveniently allows for the detection of some bacterial adhesins by haemagglutination; bacteria aggregate erythrocytes carrying the proper receptor for their adhesin.

The specificity of the interaction is shown by the ability of soluble receptor structures, often mono- or oligosaccharides, to block the adhesin-mediated adherence or haemagglutination. For example, the P fimbrial adhesin of *E. coli* recognizes Galη1–4Galβ-containing receptors on human erythrocytes, whose agglutination can be inhibited in the presence of Galη1–4Galβ-containing receptor analogues. Type 1 fimbriae in *E. coli* recognize mannose-containing glycoproteins on, e.g., horse and guinea pig erythrocytes, whose agglutination is abolished in the presence of mannose, hence termed mannose-sensitive haemagglutination.

Adherence to extracellular matrix structures

Many bacteria can adhere to extracellular matrix (ECM) structures, e.g., the proteins collagen, laminin or fibronectin. Such structures may be exposed if the epithelial layer is injured. Indeed, adherence to the extracellular matrix has been shown to promote bacterial colonisation of damaged tissues (36). If translocating bacteria reach the blood stream, adsorbtion of glycoproteins such as fibronectin onto their surface can be a strategy to avoid host defences (37, 38). *E. coli* strains isolated from patients with colonic disorders, likely to have a damaged colonic mucosa, express higher binding to extracellular matrix proteins than *E. coli* strains from healthy subjects (39).

The intestinal bacterium for which binding to matrix components is best defined is *E. coli*, which can bind to a variety of different extracellular matrix proteins. Much of this binding may be mediated through well known *E. coli* adhesins, including type 1 fimbriae, P fimbriae, S fimbriae and Dr adhesins. Type 1 fimbriae bind to oligomannoside chains on the laminin network of basement membranes (40). *E. coli* P fimbriae bind to fibronectin via a protein-protein interaction that does not involve the adhesin subunit, but rather the minor proteins PapE and PapF of P fimbriae (41, 36). S fimbriae bind to sialic acid exposed on the oligosaccharide chains of laminin (42). Dr adhesin, which is a non-fimbrial adhesin, interacts strongly with type IV collagen, which is a major component in basement

membranes (43). Curli are thin hairlike structures found on *E. coli*. They interact with matrix proteins such as fibronectin and laminin. Furthermore, many *E. coli* adhesins, including type 1, P and S fimbriae bind to the lysine-binding sites of plasminogen. *E. coli* with S fimbriae or curli have been shown to bind plasminogen and the tissue-type plasminogen activator (t-PA) (44), resulting in activation and generation of localized plasmin activity (45, 44). Plasmin digests different extracellular matrix proteins and activates collagenase, providing effective means for bacteria to invade host tissues (46, 47). Plasminogen binding is not dependent on the lectin binding subunits of fimbriae (46).

Certain strains of *Enterococcus faecalis* bind to collagen type I (48) or fibronectin (49). Both E. *faecalis* and *E. faecium* have been reported to bind to vitronectin, lactoferrin and thrombospondin (50, 51).

Staphylococcus aureus is traditionally not regarded as a resident member in the intestinal ecosystem, but recent studies of young infants indicate that these bacteria are both common and quite numerous in the early intestinal microflora (Lindberg, unpublished data). *S. aureus* express a number of surface adhesins specific for collagen, fibronectin, elastin, laminin and fibrinogen, which are thought to play an important role in pathogenicity (52–55, 38). Certain proteins seem to have a broad specificity, mediating binding to several different matrix glycoproteins (56). Binding of *S. aureus* proteins to the lysine-binding sites of plasminogen results in the formation of bacterial surface-bound enzymatically active plasmin, which could, as described above, facilitate bacterial invasion of host tissues (57).

Lactobacillus isolates from the intestinal tract or oral cavity of humans have been reported to bind collagen type I (58, 59). *L. reuterii* and several other *Lactobacillus* species adhere to fibronectin (60, 61) or to collagen type I (62).

Bacteroides fragilis binds to laminin, fibronectin and collagen (63), *Bacteroides gingivalis* to fibrinogen (64) and *Fusobacterium nucleatum* to fibronectin (65). Concerning other groups of strict anaerobic bacteria, little is known concerning their binding to extracellular matrix proteins.

Examples of adherence occurring in the normal human intestinal microflora

Escherichia coli: Duguid demonstrated that the mannose-specific haemagglutination of *E. coli* was associated with surface structures, which he termed type 1 fimbriae. Two years later, he demonstrated type 1 fimbriae on the closely related species *Shigella*, and proved that these fimbriae enabled the bacteria to bind to human and guinea-pig colonic epithelial cells (66, 67). In U.S. literature, fimbriae are often termed 'pili' after the term Brinton used to describe the same structures as Duguid 10 years later (68).

Type 1 fimbriae are found in almost all isolates of *E. coli*, pathogenic as well as commensal, as well as in other members of the *Enterobacteriaceae* family, such as *Enterobacter*, *Klebsiella*, *Shigella* and *Salmonella* (69). In addition, other Gram-negative bacteria, including *Pseudomonas* (70) and *Vibrio cholerae* (71) possess mannose-specific adhesins, other than type 1 fimbriae. Binding to mannose-containing receptors is, thus, the most common adherence specificity *so far* recognized among intestinal bacteria, and is also found among certain species of lactobacilli (see below).

The mannose-specific adhesin of type 1 fimbriated *E. coli*, termed FimH, recognizes the receptor trisaccharide Manη1–3Manβ1–4GlcNAc, found in the branching region of N-linked oligosaccharide chains (72). The adhesive protein FimH possesses a monovalent recognition site, which might best accommodate molecules with the size of a trisaccharide or those with 3 η mannosyl residues (73). Since type 1 fimbriae only recognize the η3-linked mannose residue in a terminal position, neither complex type chains (74), nor the least processed types of high-mannose oligosaccharide chains, which carry repeating Manη1–2Man units (75) are functional receptors. Type 1 fimbriae bind to human intestinal epithelial cells (67, 76, 77). The mannose-specific lectin of type 1 fimbriae also recognizes the type of carbohydrate chains that appears on secretory IgA, especially of the IgA2 subclass, which predominates in the human colon (78). Mannose does not occur on glycolipids, and it is questionable whether mucus contains a sufficient number of *N*-linked oligosaccharide chains of the right type as to function as physiologically relevant receptors for type 1 fimbriae.

Receptors for type 1 fimbriae, i.e., mannose-containing glycoproteins, occur in abundance after mucosal injury, because the newly synthesized immature intestinal epithelial cells carry more glycoproteins with terminal mannose (79, 80). In this context, it is interesting to note that starvation of experimental animals leads to a massively increased adherence of type 1-fimbriated *E. coli* to the intestinal mucosa, which secondarily leads to increased translocation over the intestinal barrier (81). Whether this is a result of altered receptor conformation, or altered adhesin expression by intestinal bacteria remains to be shown.

Type 1 fimbriae have not been linked to long-term persistence in the human gut. A majority of both resident and transient strains seem to be capable of expressing type 1 fimbriae (82–84). In neonatal rats colonized with an *E. coli* K1 isolate, the depletion of the *fimA* gene coding for the type 1 fimbrial major subunit, impedes oropharyngeal but not intestinal colonisation (85). Disruption of *fimA* decreased transmission of *E. coli* between littermates dramatically (86). In gnotobiotic adult rats, a down-regulation of type 1 fimbrial expression occurs some days after colonisation (87, 88). Still, binding of *E. coli* to secretory

IgA, probably via its mannose oligosaccharides, plays a role in intestinal colonisation, since *E. coli* recovered from people with selective IgA deficiency less often carry the gene for type 1 fimbriae and express less mannose-specific adhesins per cell compared with *E. coli* recovered from control individuals (89). In *E. coli* recovered from IgA-deficient individuals, other adhesins than type 1 fimbriae, such as S fimbriae and P fimbriae, seem to be increased in frequency, maybe as a compensatory mechanism (Friman, unpublished data). Type 1 fimbriae may be most important in initial stages of colonisation. Krogfeldt et al. noted an upregulation of type 1 fimbriae in bacteria when establishing in the mouse large intestine (90) and a type 1-fimbriated *E. coli* strain was a better colonizer of the mouse intestine that its adhesin-negative counterpart in a short-term experiment (91).

P fimbriae were identified as a virulence factor for pyelonephritis. The name derives from this association (pyelonephritis), and from their capacity to agglutinate human blood cells of the P blood group. Their receptor was later identified as the Galη1–4Galβ sequence, which occurs on glycolipids in human urinary tract epithelial cells and human erythrocytes (92). Urinary tract isolates derive from the intestinal microflora, and P fimbriae also bind to small (77) and large (76, 77) intestinal epithelial cells of humans and rats (87).

P fimbriae are the adhesins that have been most convincingly linked to persistence in the intestinal microflora. In epidemiological studies, resident *E. coli* strains more often than transient ones express P fimbriae (82–84) or carry the genes enabling their synthesis (Nowrouzian, unpublished data). In gnotobiotic rats, *E. coli* possessing P fimbriae colonize much better than the isogenic counterpart lacking these adhesins (87), and bacterial P-fimbrial expression seems to be retained during intestinal colonisation (87, 88). On the other hand, a P-fimbriated *E. coli* in which the tip adhesin was mutated retained its ability to colonize the monkey intestine (93) as well as the rat intestine (Herías, unpublished data).

E. coli S fimbriae recognize terminal sialic acid on sialyl oligosaccharide chains and are associated with urinary tract infection and neonatal sepsis/meningitis (94, 95). S fimbriae-mediated adherence of *E. coli* O18 to cellular fibronectin is inhibited by neuraminidase treatment or by incubation of the bacteria with sialyl η-2–3lactose (96). Dr haemagglutinin, which binds to an epitope on the peptide chain of the complement-regulating protein DAF, is found in *E. coli* isolates from urinary tract infections (97, 98). S fimbriae and Dr haemagglutinin both mediate attachment to human intestinal epithelium (99, 77). However, these adhesins are not more frequently found among resident than transient *E. coli* (Nowrouzian, unpublished data) and S fimbriae do not contribute to colonisation in the gnotobiotic rat model.

In intestinal bacteria other than *E. coli*, adhesins with defined specificities have rarely been described. When adherence has been studied, a very limited number of strains (< 10) have been examined. Thus, with the exception of human lactobacilli (10), no systematic studies have been performed regarding the proportion of intestinal isolates that can adhere to intestinal epithelial cells or other mucosal components. We therefore do not know the ecological significance of adhesins in these bacteria. Clearly, much more detailed studies are needed in order to elucidate the role of adherence in colonisation and persistence for other members of the intestinal microflora than *E. coli*.

Enterococci constitute a major part of the facultatively anaerobic intestinal population in adults as well as infants. Enterococci have been shown to adhere to urinary tract epithelial cells (100), heart valves (100) and renal tubular cells (101). The only identified receptor structure so far is the connective tissue component fibronectin (102, 103). In an intestinal overgrowth model in mice, *Enterococcus faecalis* cells were seen to adhere to epithelial cell microvillus membranes in the mouse ileum, caecum and colon (104, 105), but their capacity to adhere to human intestinal mucosa has not been studied.

Lactobacilli are part of the normal flora of the small and large intestine. Lactobacilli have been shown to be associated with the human intestinal mucosa *in vivo* (106) and may bind to freshly isolated human ileal (107) and colonic (108) cells. Binding to human intestinal cell lines has been shown for members of *Lactobacillus acidophilus* (109, 110), *Lactobacillus rhamnosus* (111), and other lactobacilli (112), although no receptor structures were defined. Strains of *Lactobacillus plantarum* isolated from the human gastrointestinal tract express a mannose-binding adhesin (108, 10). This adhesin was expressed by a majority of mucosal isolates of *Lactobacillus plantarum*, whereas most other *Lactobacillus* groups were negative for adherence to the HT-29 cell line (10). Since these lactobacilli were retrieved from mucosal biopsies or from scraping the base of the tongue, it is clear that lactobacilli may colonize the human mucosa despite lack of demonstrable adherence *in vitro*. Fuller observed that *Lactobacillus* adherence to chicken crop epithelium was advantageous, but not a prerequisite, for colonisation (113). Moreover, *in vitro* adherence to isolated gastrointestinal epithelial cells did not predict *in vivo* association with the mucosa (114). Autoaggregation has been noted frequently among freshly isolated intestinal lactobacilli from pigs (115) and humans (Ahrné, unpublished data). It is possible that the autoaggregated stage is advantageous for intestinal lactobacilli, as has been demonstrated for bacteria in other ecosystems.

Fimbriated isolates of *Bacteroides fragilis* and *Bacteroides ovatus* adhere to intestinal epithelial cell lines (116, 117). *B. vulgatus* (118) and *B. fragilis* (119) agglutinate erythrocytes from various species and autoagglutination has been demonstrated in *B. fragilis* (120). Fimbriae are

more often expressed by intestinal isolates of *B. fragilis* or *B. melaninogenicus* than by isolates of these same organisms cultured from blood (121). *B. fragilis* can adhere to the connective tissue component laminin, in contrast to isolates of the species *B. thetaiotaomicron* and *B. ovatus* (122).

Some strains of bifidobacteria adhere to the Caco-2 cell line (123). Of strains recovered from infant faeces, all seven *Bifidobacterium bifidum* strains were adherent, autoagglutinating, hydrophobic and haemagglutination positive (124). In contrast, 3/3 *B. breve* strains, 3/3 *B. adolescentis* strains and one each of *B. pseudolongum* and *B. infantis* were negative with respect to adherence, autoagglutination and haemagglutination (124). Binding of bifidobacteria to the glycolipid asialo-GM1, the same receptor as utilized by CFAI-carrying enterotoxigenic *E. coli* (125), has been demonstrated (126).

Examples of adherence occurring in potentially pathogenic bacteria: different pathovars of E. coli and Clostridium difficile

Attachment is the first step in the pathogenesis of many bacterial infections. Protein-carbohydrate interactions appear to play a critical role in the adherence of pathogens to epithelial surfaces. Binding to epithelial cells may also activate a complex signal transduction cascade in the host cell, leading to an inflammatory response, as well as the expression of new genes in the bacterium that are important in the pathogenic process (127, 128).

Bacteria causing gastrointestinal infection need to penetrate the mucus layer before attaching themselves to intestinal epithelial cells. Among the enteric pathogens, we review here the mechanisms of adherence of diarrhoeagenic *Escherichia coli* and *Clostridium difficile*. As described above, *E. coli* is the predominant facultative anaerobe of the human colonic flora. However, certain *E. coli* strains have evolved the ability to cause diarrhoeal disease (129). The diarrhoeagenic *E. coli* are divided into 6 major categories differentiated on the basis of pathogenic features (129, 130). The most highly conserved feature of diarrhoeagenic strains is their ability to colonize the intestinal mucosal surface despite competition for nutrients by the indigenous intestinal flora (129, 131). *C. difficile* is not a significant component of the colonic microflora in healthy adults humans, but it can establish large populations in infants before they acquire a complete flora (132, 133). In adults, *C. difficile* is the etiological agent of antibiotic-associated pseudomembranous colitis and of most cases of colitis or diarrhoea in patients undergoing antibiotic therapy (134). Infection is associated with antibiotic use because the normal gut microflora has to be disrupted before *C. difficile* can establish itself at high numbers and produce toxins (135, 136).

Enterotoxigenic *E. coli* (ETEC) adhere to the small bowel enterocytes by adhesins called colonisation factors (CFAs) and cause diarrhoea through the action of enterotoxins termed LT (heat labile) and ST (heat stable) (137). ETEC fimbriae confer the species specificity of the pathogen. Thus, human ETEC strains express adhesins termed CFAs, calf strains K99 and pig strains K88. At present 20 different CFAs, classified as fimbriae or fibrillae based on their morphology, have been described in ETEC causing diarrhoea in humans (138). Epidemiological studies suggest that CFA/I, CFA/II or CFA/IV are expressed by approximately 75% of human ETEC strains worldwide (139). Fimbrial gene clusters, containing a series of genes which encodes the major fimbrial protein subunit and accessory proteins, have been described (140, 141). Receptors for CFAs are glycoconjugates present on eukaryotic cell membranes (142). Subcomponents of CFA/II and CFA/IV bind to asialo GM1 (125) and the subcomponent CS3 of CFA/II bind to GalNAcβ1–4gal-containing receptor structures (143).

Enteropathogenic *E. coli* (EPEC) diarrhoea is not mediated by toxins. EPEC is a class of diarrhoeagenic *E. coli* identified by their ability to cause attaching and effacing (A/E) lesions on intestinal cells (144). This histological lesion is characterized by effacing of microvilli brush border and intimate adherence between the bacterium and the epithelial cell membrane (145, 146). The resulting tight association is accompanied by cytoskeletal changes in the epithelial cell, including accumulation of polarized actin and formation of paedestal like structures (144). The interaction between EPEC and host cells have been divided into three stages: initial adherence, signal transduction and intimate attachment (147–149). The initial adherence to cultured epithelial cells *in vitro* is mediated by type IV fimbriae known as 'bundle-forming pili' (BFP) (150). Recently it has been demonstrated that BFP mediate bacterial-bacterial interactions in a human intestinal organ culture model (151). BFP is encoded by a 50 to 70 *MDa* plasmid, known as the EAF plasmid. The second stage in the interaction involves the secretion of several effector proteins (EspA, EspB, EspD) by a type III secretion system. Signal transduction events occur within the host (152), including Ca^{2+} release from internal stores, activation of phospholipase C (PLC), protein kinase C (PKC) and inositol triphosphate (IP_3) fluxes. In the third stage of infection the intimate adherence of the bacteria to the epithelial cells is mediated by a 94 to 97 kDa outer membrane protein (OMP) called «intimin». This outer membrane ligand binds to a receptor (Tir) which has recently been found to be of bacterial origin (153). Intimin can also bind β1-integrins (154). All the genes necessary for the formation of A/E lesions by EPEC, including the gene encoding intimin (*eae*), are located within a 35 kbp pathogenicity island called LEE (locus of enterocyte effacement) (155). This region is not present in *E. coli* strains in the normal flora.

Several *in vitro* studies have identified oligosaccharide structures that are potential receptors for EPEC: GalNAc (156), fucosylated milk oligosaccharide sequences (157), GM3 gangliosides (158), GalNacβ1–4Gal portion of asialo-GM1 and asialo-GM2 structures (159) and asialo-lactosamine sequences of *N*-linked glycoproteins (160). The studies of Vanmaele et al. demonstrated that *N*-acetyl lactosamyl-BSA (Galβ1–4GlucNAc), followed by Lewis X-BSA (Galβ 1–4 Fucη 1–3 GlcNAc) were the most effective inhibitors of EPEC attachment to Hep-2 cells. Furthermore, the interactions of bacteria with these specific glycoconjugates cause a decrease in the expression of BfpA, the structural subunit of BFP and intimin. These results suggest that the glyconjugate inhibition of EPEC binding to cells might be achieved, wholly or in part, by an active mechanism that is distinct from simple competitive antagonism of receptor-adhesin interactions (161).

The term Enterohaemorragic *E. coli* (EHEC) was coined to denote strains that cause haemorrhagic colitis (HC) and haemolytic uremic syndrome (HUS), express shiga-like-toxin, produce A/E lesions on epithelial cells and possess a ca. 60 *MDa* plasmid (130). The classic A/E histopathology has been seen in gnotobiotic piglets, infants rabbits and cultured epithelial cells infected with *E. coli* O157:H7 (129). These A/E lesions resemble those produced by EPEC strains. The 94 to 97 kDa OMP intimin is the only bacterial adherence factor identified to date that has been demonstrated important for intestinal colonisation in animal models (162). As reported above for EPEC, the *eae* gene (encoding intimin) lies within the 35kb LEE pathogenicity island in strains associated with human disease (162). Other adherence factors distinct from intimin have been suggested to play a role in the adherence of serotype other than O157:H7 to epithelial cells, but no specific candidate adhesins have been identified (129).

Enteroaggregative *E. coli* (EAEC) pathotype is defined by aggregative adherence (AA) to Hep-2 cells, where bacteria display adherence to the cell surface and also to the intervening substratum in a stacked-brick configuration (163). EAEC strains characteristically enhance mucus secretion with trapping of the bacteria in a bacterium-mucus film; the ability of EAEC to bind mucus has been demonstrated *in vitro* (164). The AA phenotype is associated with the presence of a plasmid 60 to 65 *MDa* in size and with the expression of one or two distinct aggregative adherence fimbriae (AAF/I and AAF/II) (165, 166). Recently it has been reported that the genes encoding the Pet and EAST (enteroaggregative heat stable enterotoxin) enterotoxins and the proteins related to the AAF/II biogenesis are located in a 23 kb region on the 65*MDa* virulence plasmid (167). The relevance of adherence factors other than AAF remains to be demonstrated.

An exact pathogenic scheme of Enteroinvasive *E. coli* (EIEC) has yet to be elucidated. However, studies on EIEC suggest that its pathogenic features are virtually identical to those of *Shigella* spp. (168). The current model of *Shigella* and EIEC pathogenesis comprises: (i) epithelial cell penetration; (ii) lysis of endocytic vacuole; (iii) intracellular multiplication; (iv) directional movement through the cytoplasm; and (v) extension into adjacent epithelial cells (169). This sequence of events is followed by a strong inflammatory reaction. A 140 *MDa* plasmid, named pInv, carries the genes necessary for invasiveness, including the loci encoding a type III secretion apparatus and the Ipa protein effectors of the invasive phenotype (170).

Diffusely adherent *E. coli* (DAEC) is a category of potentially diarrhoeagenic *E. coli* defined by diffuse adherence (DA) to Hep-2 cells, where bacteria are dispersed over the surface of the cell with little aggregation and little adherence to the intervening substratum (163). DAEC strains are able to induce finger-like projections extending from the surface of infected Caco-2 or Hep-2 cells in which bacteria are embedded (171). A surface fimbria and a 100 kDa outer membrane protein, which are associated with the DA phenotype, have been described (172).

Our understanding on the pathogenesis of enterodiarrhoeagenic *E. coli* has made remarkable progress in recent decades. These pathogenic *E. coli* display a large versatility in their ability to adhere to the epithelial cells. Several different pathogen-specific adhesive factors have been discovered; however, many questions regarding pathogenic *E. coli*/host cell interactions remain to be answered.

Clostridium difficile The major virulence factors in *C. difficile* are the toxins A and B, but other factors are implicated in the colonisation process such as adherence factors and hydrolytic enzymes (173–176, 21). Although a minority of strains of *C. difficile* have been found to carry a small number of fimbriae, no definitive role in the colonisation process for these structures can be ascribed at the moment (177). No salient genes encoding fimbriae have been identified on the genome sequence of *C. difficile* 630 (Wren, unpublished data). *C. difficile* cell surfaces are relatively hydrophobic and their net charge is positive (178). This might facilitate interactions with negatively charged host cells and, thus, contribute to gut colonisation.

It is clearly established that *C. difficile* can associate with the intestinal mucosa in man (179) and animals (180, 181, 21, 182). In the hamster model, a highly virulent toxigenic strain adhered better than an avirulent non-toxigenic one. However, co-administration of toxin A with the non-toxigenic strain substantially raised adhesion, suggesting that adhesion is facilitated by toxin A-mediated damage (180). These data have been recently confirmed using an '*in vivo*' and an '*ex vivo*' model of axenic mice to study adhesion to or association with caecal mucus (182).

C. difficile can adhere to tissue culture cells of intestinal origin such as Caco-2 cells and mucus-producing HT-29 cells, but also to cell lines of other origin (Vero, HeLa,

KB). The bacteria interact with the Caco-2 brush-border microvilli on the mucosal surface and strongly bind to the mucus layer that covers the surface of HT29-MTX cells. In aerobiosis, the adherence process is increased after heat shock (181, 21). A gene encoding a putative adhesive protein was cloned and the recombinant clone adhered to Vero, Caco-2, KB and HeLa cells and to mucus isolated from axenic mice (21). The adherence was blocked by anti *C. difficile* antibodies, by a surface extract of *C. difficile* and by mucus isolated from axenic mice.

Adherence inhibition studies revealed that glucose, galactose and Gal-NAc, and oligosaccharides containing these sugars partially block adherence to tissue culture cells, suggesting that the adhesin is a lectin. In the same way, the study of Naaber et al. (183) demonstrated that both xylitol and colostrum inhibited the adhesion of *C. difficile* to Caco-2 cells. Inhibition by xylitol was dose dependent. Inhibition of adherence is one possible way to develop treatment and prophylaxis against *C. difficile* infections.

The adhesion of *C. difficile* to Caco-2 cells significantly increased following treatment aimed at opening intercellular junctions, suggesting that *C. difficile* adheres to the basolateral surface of Caco-2 cells. Moreover, a 36kDa surface protein might play a role in the adherence process (184, 185). A relationship between the presence of a crystalline surface layer (S-layer) and bacterial adherence to HeLa cells has been observed in a *C. difficile* strain: Fab fragments prepared from antisera against the S-layer protein subunits partially inhibited adhesion of the bacterium to HeLa cells (186).

Although the role of antibiotic exposure as a predisposing factor to *C. difficile* colonisation and infection is well established, the exact mechanisms by which the colonisation process is achieved is still unclear and need further experiments.

As evident from above, there is no simple relationship between *in vitro* adherence capacity to, e.g., intestinal cells and colonising capacity. Thus, some *E. coli* strains present for years in the intestinal microflora of an individual may lack adhesive potential *in vitro* (83). Similarly, many lactobacilli isolated from human mucosal biopsies are virtually non-adherent to an intestinal cell line (10) and a *Lactobacillus rhamnosus* strain which was able to persist in experimentally colonized individuals (187) is non-adherent to the colonic cell line HT-29 (Adlerberth, unpublished data). Several reasons may account for this: firstly, strains carrying genes for adhesins may not express these adhesins when cultured *in vitro*. Thus, P fimbriae and S fimbriae in *E. coli* are expressed by approximately 50% of genetically positive isolates (Nowrouzian, unpublished data). Secondly, the bacterium may carry adhesins recognising receptors not present on the cell line used, but which are present on enterocytes or in mucus *in vivo*. In summary, there is only one way to proceed if one wants to isolate strains with superior colonising capacity: to test them in administration studies using human volunteers. Thirdly, other bacterial factors than adherence may play a decisive role in the ability of bacteria to persist in the colonic microflora. Such factors include capsule formation (88), synthesis of the iron-trapping compounds such as aerobactin and a smooth LPS (188). Capsules and LPS render the bacteria hydrophilic and negatively charged which has been suggested to prevent their entrapment in mucus (189). Metabolic functions are, in addition, likely to play a decisive role in the colonisation process.

BACTERIAL PROPERTIES FACILITATING COLONISATION AND HOST DAMAGE: METABOLIC ENZYMES

One way for the intestinal microbiota to influence its environment is by producing hydrolytic enzymes. Such enzymes can be either cell-bound or extracellular (190) and hydrolyse oligosaccharides, peptides, and aliphatic lipids or steroids (191). Usually, only minor populations of intestinal bacteria possess a certain hydrolytic capacity. For example, only a subpopulation of bacteria, about 1% of faecal cultivatable bacteria (192), is responsible for the major degradation of the oligosaccharide chains of gut mucin glycoproteins (190).

Role of glucidolytic enzymes

Direct mechanisms: cleavage of oligosaccharidic chains of mucins. Mucins are the most important structural components of the mucus layer that covers and protects the intestinal mucosa. Mucins are glycoproteins composed of a peptidic core with an abundance of attached *O*-linked oligosaccharidic chains. Many glucidolytic enzymes are involved in mucin degradation. In the first step, terminal sugars conferring the blood group specificity are removed (fucose, η-galactose, *N*-acetylgalactosamine). During this process sialic acids, β-galactose and *N*-acetyl glucosamine are exposed, which may then be cleaved off sequentially until only the protein core is left (193).

Mucin-degrading enzymes are produced by the endogenous microflora in the large gut (190). *Clostridium perfringens*, *Bacteroides fragilis*, *Ruminococcus torques* and *Bifidobacterium bifidum* are well known to display a high mucinolytic activity. They produce extracellular specific glycosidases which cleave monosaccharides (galactose, *N*-acetylgalactosamine, sialic acid) from oligosaccharidic chains (194, 195). Free monosaccharides function as nutrients for the human colon ecosystem and the mucin-degrading glycosidases leads to structural and functional changes in mucins. These changes can lead to a destruction of receptors for some microorganisms or toxins or to an unmasking of receptors buried in the mucus layer (196). By the production of nutrients and unmasking or destruction of receptors, bacterial modifications of mucins could

influence colonisation and maintenance of the indigenous intestinal microbiota.

Indirect mechanisms: induction of expression of enzymes by bacteria or the host. Studies of germ-free mice have revealed that members of the autochthonous flora can interact with the intestine to influence specific biochemical pathways in the host.

Addition of epithelial cell-derived mucins to an established continuous-flow culture of anaerobic isolates from a human caecal flora belonging to the genera *Lactobacillus*, *Bifidobacterium*, *Propionibacterium*, *Clostridium* and *Streptococcus* as well as coliforms and *Bacteroides fragilis* markedly increased the levels of secreted bacterial η- and β-glycosidases. The augmentation of glycosidases was associated with enhanced growth (197).

Umesaki et al. found that colonisation of germ free Balb/c mice with a suspension of faecal microorganisms obtained from conventional mice leads to the induction of a η1,2-fucosyltransferase activity in the epithelium (198). Intestinal colonisation with segmented filamentous bacteria, which are indigenous intestinal bacteria strongly bound to the ileal epithelial cells, induces the activation of intraepithelial lymphocytes but also the expression of fucosyl asialo GM1 glycolipid, major histocompatibility class II molecules and the enhancement of cryptal cell proliferation of the small intestine. The function of asialo GM1 is not well known. It could act as a receptor for some bacteria and the fucosylation of this glycolipid could mask the receptor site for these bacteria (199).

Bry et al. have observed that conventional and germ free NMRI mice are able to initiate production of Fuc η1,2 Galβ-containing glycoconjugates in their distal small intestine. However, in the absence of microflora, the capacity to produce these fucosylated glycoconjugates is lost 25–28 days after birth. Inoculation of adult germ free NMRI mice with a conventional flora reinitiates production of fucosylated glycoconjugates. Conventionalisation of germ free mice results in transcriptional activation of a host η1,2 fucosyltransferase gene in the ileum. Reverse transcriptase polymerase chain reaction (RT-PCR) of cellular duodenal, ileal and colonic RNA from germ-free and ex germ-free mice confirms that production of fucosylated glycoconjugates is associated with accumulation of host-η 1,2-fucosyltransferase mRNA (200).

The ability of *Bacteroides thetaiotaomicron* to induce production of fucosylated glycoconjugates has been studied. It is linked to its capacity to utilize fucose as a carbon source. Fu 4 is a *B. thetaiotaomicron* mutant strain that lacks the ability to utilize L-fucose and D-arabinose as carbon sources. The Fu 4 strain is unable to reinitiate production of host fucosylated glycoconjugates. The fact that a strain unable to utilize fucose cannot neither induce production of host fucosylated glycoconjugates suggests that a fucose metabolite may be involved in mediating some aspects of this host-microbial interaction. The ability to produce enzymes and to induce production of its own nutrients would provide a selective advantage to a microorganism competing with other occupants for a place in an ecosystem with limited resources (200).

B. thetaiotaomicron must reach a critical population density (10^4 CFU/ml) to induce production of fucosylated glycoconjugates in the intestine of NMRI mice (200). No binding of bacteria to epithelial cells was observed after inoculation of germ-free mice with a wild-type strain. These results suggest that the induction is the result of secretion of a soluble microbial factor dependent on bacterial density. The term «quorum-sensing» describes the phenomenon of linking bacterial cell density to expression of a particular metabolic process (201) (see a separate review by Swift et al. in this supplement).

Examples of role of glucidolytic enzymes in intestinal colonisation. Bacteroides ovatus produces at least three η galactosidases (I, II and III). η galactosidases I and II hydrolyse simple η galactosides such as melibiose, raffinose, stachyose and partially guar gum, whereas η galactosidase III hydrolyses melibiose only. When *B. ovatus* η galactosidase III mutant and wild type strains are mixed and inoculated in germfree mice, the mutant is outcompeted by the wild type strain (202). The locus which contains η galactosidase III may be important for colonisation *in vivo*. However, the η galactosidase III activity is very weak compared to the η galactosidases I and II activity when *B. ovatus* is grown *in vitro*. One of the explanations is that η galactosidase III could be an inducible enzyme and the optimal inducer is not yet known.

Production of glycosidases can be enhanced when bacteria grow in culture medium supplemented with porcine gastric mucin. This phenomenon has been observed with *Streptococcus oralis* (203) and *B. ovatus*, which produce η galactosidase IV in presence of mucin. This is surprising, since η galactoside residues are only minor components of mucins. This could indicate that *B. ovatus* is highly adapted to utilisation of galactosides from any available substrate in the digestive tract (202).

B. thetaiotaomicron can ferment host-derived polysaccharides such as chondroitin sulfate (CS), heparin (HP) and hyaluronic acid. The pathways for utilisation of CS and HP seemed to be independent of each other but a gene termed *chuR* was identified that provided a link between these two utilisation systems. This *chuR* gene is probably a regulatory gene. Some of the genes controlled by *chuR* (like those that permit *B. thetaiotaomicron* to utilize CS and HP) are important for the survival of this bacterium in the digestive tract. A *chuR* mutant strain that lacks the ability to utilize chondroitin sulfate and heparin is unable to compete with the wild type for colonisation of the intestinal tract of germ-free mice. When the authors introduced a second mutation in the *chuR* mutants to obtain suppressor mutations, they restored the ability of a *chuR* disruption mutant to utilize CS and HP. Unlike *chuR*

mutant, suppressor mutants coexist with the wild type strain in the murine intestinal tract (204, 205).

Example of role of glucidolytic enzymes in colonisation and colonisation resistance. C. difficile produces enzymes able to hydrolyse chondroitin sulfate, hyaluronic acid and, to a lower extent, heparin (206). A simplified *in vivo* model of anti *C. difficile* barrier flora has been obtained in trixenic mice. Three species are involved in this barrier effect: *Clostridium indolis*, *Clostridium cocleatum* and *Clostridium fusiformis*. *C. indolis* produces a sialidase activity as its only mucinolytic activity. In contrast, *C. cocleatum* displays much higher and varied mucin-degrading activity: η galactosidase, β galactosidase, β glucosidase, β-*N*-acetylglucosaminidase, sialidase and η-*N*–acetylgalactosaminidase (207). It has been suggested that the sialidase activity expressed by *C. indolis* could play a role in the colonisation process by creating a specific niche for *C. cocleatum*. In fact, *C. cocleatum* is able to colonize the digestive tract of the mice only after the implantation of *C. indolis*. Similarly, *C. cocleatum* with its high glucidolytic activity creates a niche for *C. fusiformis*. In this simplified model, the implantation of the strains in the digestive tract is sequential: the first strain capable to colonize is *C. indolis*, then *C. cocleatum* and last *C. fusiformis*. The colonisation resistance could be due to a competition between *C. fusiformis* and *C. difficile* for nutrients or receptor sites. The sialidase genes from *C. indolis* and *C. cocleatum* have been cloned to try to understand their role in the colonisation process (208).

Role of proteolytic enzymes

Many bacteria produce large amount of various proteolytic enzymes with broad substrate specificity (209–211). Bacteria need nutrients such as amino acids or oligopeptides for growth. By furnishing nutrients for the bacteria, proteolytic enzymes could facilitate intestinal colonisation.

Some bacteria produce proteases and peptidases, which degrade large proteins such as casein and bovine serum albumin into short peptides and free amino acids. This has been described, for instance, in lactic acid bacteria (212, 213). *In vitro* studies have shown that casein and bovine serum albumin are partly degraded in human faeces over a 96 h-incubation period. The products are soluble peptides, ammonia and volatile fatty acids. The predominant proteolytic bacteria in the faecal samples were identified as *Bacteroides sp.* and *Propionibacterium sp.* Other proteolytic bacteria belong to the genera *Streptococcus*, *Clostridium*, *Bacillus* and *Staphylococcus* (214).

Macfarlane et al. demonstrated that a substantial population of the proteolytic activity in normal faeces was of bacterial origin. The faecal proteolysis was both qualitatively and quantitatively different from that of the small intestine (215). Indeed, different proteolytic activity inhibitors such as iodoacetate, EDTA or cysteine significantly inhibit proteolysis in faeces but not in the small intestinal contents, showing that cysteine and metalloproteases produced by bacteria in the colon are important sources of proteolysis in addition to pancreatic enzymes. Cell-bound proteases were found in *B. fragilis*, whereas extracellular proteases are synthesized by *Enterococcus faecalis*, *Propionibacterium acnes*, *Clostridium perfringens*, *Clostridium bifermentans* and *Clostridium sporogenes*.

Role in evading human immune system. IgA1 proteases have been described in different bacteria. These include colonizers of the upper respiratory tract involved in meningitis: *H. influenzae* (216), *Neisseria meningitidis* (217) and *Streptococcus pneumoniae* (218) as well as species involved in urogenital infections: *Neisseria gonorrhoeae* (219) and *Ureaplasma urealyticum* (220).

Cleavage of IgA1 by IgA1 proteases could allow the bacteria to evade human immune system facilitating mucosal colonisation (221). The role of IgA1 proteases for bacteria persistence in the colon has not been studied. The dominant IgA isotype in colonic secretions is IgA2, which cannot be cleaved by most IgA proteases.

Role in invasion or demasking receptors. The role of proteolytic enzymes in colonisation has been well studied in *Porphyromonas gingivalis*. This bacterium plays a major role in periodontal disease.

Kontani et al. have shown that a cysteine protease enhanced the binding of purified *P. gingivalis* fimbriae to the cell surface of monolayered fibroblasts and matrix proteins. Degradation of matrix proteins by the protease exposed arginine residues, to which *P. gingivalis* fimbriae could bind effectively (222).

Tokuda et al. have demonstrated that a cysteine protease mutant of *P. gingivalis* displayed reduced interaction with Gram-positive bacteria, immobilized extracellular matrix proteins, type I collagen and human epithelial cells (223, 224). Thus, the proteases of *P. gingivalis* could play a major role in colonisation.

Toxic activity. Numerous proteolytic enzymes have been described in *Clostridium*. Schiavo et al. have shown that tetanus and botulinum toxins serotype B are metalloproteases. These proteolytic enzymes are able to cleave synaptobrevin, which is an integral membrane protein in small synaptic vesicles, hence blocking neurotransmittor release (225). By the same way, *Clostridium sporogenes* produces an haemorrhagic toxin, which is a collagenase that hydrolyses type III and IV collagens, major components of the tunic intra and media of blood vessels, causing disruption of the vessel wall (226). Jin et al. have characterized a metalloprotease in *Clostridium perfringens* named lambda toxin. This enzyme hydrolyses different substrates such as collagen, fibronectin, fibrinogen, IgA and the complement C3 component. In mice, it can induce an increase in vascular permeability and haemorrhagic oedema (227).

Other proteolytic enzymes have been described in *Clostridium* but their role in virulence or colonisation has

not been demonstrated. For example, *C. histolyticum* produces at least seven collagenases which have been purified and characterized (228). Finally, a protease activity has been described in *Clostridium difficile* but its role in colonisation has not yet been elucidated (229).

CONCLUDING REMARKS

The colonisation of the digestive tract is a complex phenomenon associating several partners such as the bacterial population, the host and the environmental factors, which interact between each other. One of the first steps of colonisation could be adhesion between bacteria and host tissues. This phenomenon implicates non-specific and specific interactions. Several microbial surface components are involved in the specific adherence mechanisms by recognising adhesive matrix molecules on the host. Other bacterial factors may play a decisive role in the ability of bacteria to persist in the colonic microflora. Such factors include the capsule, iron trapping compounds, flagella and metabolic enzymes. Glycolytic and proteolytic enzymes are likely to play an important role in the colonisation process and the maintenance of the indigenous intestinal microflora. These enzymes act in a complex way including direct mechanisms on the host components and indirect mechanisms by inducing enzyme or protein synthesis in the host. These interactions between bacteria and the host implicate the release of soluble factors dependent also of bacteria-bacteria communication. Much work has to be performed at the molecular level to understand these complex cross-talks.

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