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MiniReview

Anti-adhesion therapy of bacterial diseases: prospects and problems

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Abstract

The alarming increase in drug-resistant bacteria makes a search for novel means of fighting bacterial infections imperative. An attractive approach is the use of agents that interfere with the ability of the bacteria to adhere to tissues of the host, since such adhesion is one of the initial stages of the infectious process. The validity of this approach has been unequivocally demonstrated in experiments performed in a wide variety of animals, from mice to monkeys, and recently also in humans. Here we review various approaches to anti-adhesion therapy, including the use of receptor and adhesin analogs, dietary constituents, sublethal concentrations of antibiotics and adhesin-based vaccines. Because anti-adhesive agents are not bactericidal, the propagation and spread of resistant strains is much less likely to occur than as a result of exposure to bactericidal agents, such as antibiotics. Anti-adhesive drugs, once developed, may, therefore, serve as a new means to fight infectious diseases.

Keywords: Cranberry; Carbohydrate; Fimbria

1. Introduction

Anti-adhesion therapy and anti-adhesin immunity are meant to reduce contact between host tissues and pathogens, either by prevention or reversal of adhesion of the infectious agent. It is well established that adhesion of enteric, oral and respiratory bacteria is required for colonization and for subsequent development of disease [1]. Moreover, bacteria assume a significantly greater resistance to clearance by normal cleansing mechanisms and to killing by normal immune factors, bacteriolytic enzymes and antibiotics when they are adherent to surfaces. Such bacteria are better able to acquire nutrients, further enhancing their ability to survive and infect the host [2]. Thus, the adherent state is advantageous for bacterial survival and a key step in pathogenesis. Therefore, prevention of adhesion at an early stage following the exposure of the host to pathogens should prevent the disease.

Bacteria can adapt to many noxious or deleterious agents, either by mutation, by acquisition of new genetic material via horizontal transfer or by phenotypic variation [1]. Bacteria resistant to anti-adhesion agents may also be expected to emerge, but because these agents do not act by killing or arresting growth of the pathogen, as, for example, antibiotics do, it is reasonable to assume that strains resistant to anti-adhesion agents will be diluted with the sensitive bacteria whose adhesion is inhibited and are shed out of the host (Fig. 1). It follows that spread of bacteria resistant to the anti-adhesion agent is expected to occur at significantly lower frequencies than that of bacteria resistant to antibiotics. This would potentially allow sensitive and resistant organisms to propagate and be transmitted at equivalent rates, dramatically slowing the emergence of a predominantly resistant population.

The major drawback of anti-adhesion therapy is that most pathogens possess genes encoding for more than one type of adhesin, so that during the infectious process the population of pathogens may express more than one of these adhesins. Adhesion may also involve factors other than just adhesin–receptor interactions such as hydrophobic and other non-specific interactions under different

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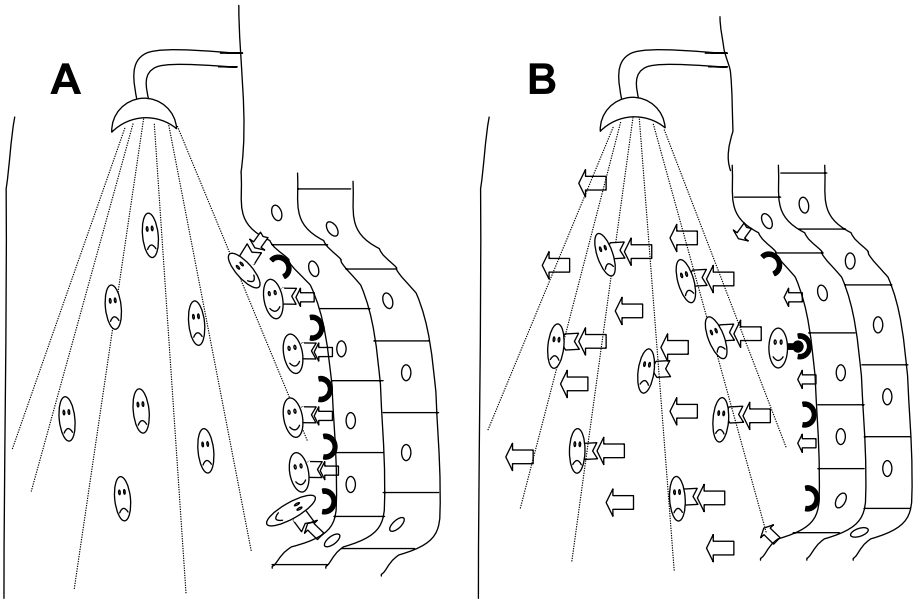

Fig. 1. Microbial adhesion and anti-adhesion therapy. Bacteria adhere via their adhesins to cognate receptors on epithelial cells of the mucosal surfaces to withstand normal cleansing mechanisms aimed at eradication of the invading pathogen. Bacteria that lack the adhesins are swept away (A). In the presence of inhibitors of adhesion (B), the bacteria are eradicated by the normal cleansing mechanism. Notice that the adhesion of a mutant with an adhesin specific for a distinct receptor is not inhibited and continues to adhere to the epithelial cells. In only a few patients would the mutant establish colonies, whereas the majority of the treated patients would continue to shed the bacteria, therefore slowing down the spread of resistant strains.

shear-forces. For anti-adhesion therapy to be effective, it will probably be necessary to use multiple agents specifically inhibiting each type of adhesin of the infecting pathogen or a single agent that exhibits a broad spectrum of anti-adhesion activity. For earlier discussions of anti-adhesion therapy see [2–5].

2. Receptor analogs as anti-adhesive agents

There is evidence that receptor analogs as agents for anti-adhesion therapy would be practical primarily against pathogens that bind to animal cells via carbohydrate-specific adhesins (i.e. lectins). In these cases, the receptor analogs are saccharides that are structurally similar to those of the glycoprotein or glycolipid receptors for the adhesin and, therefore, act by competitive inhibition. It was less than three decades ago that mannose was first shown to be a receptor for enterobacteria [6]. Since then, the sugar specificities of many bacteria have been determined, leading to the development of receptor-like carbohydrates, which inhibit the adhesion of pathogens to host cells and tissues [1]. The concentrations of the carbohydrates required for effective inhibition of adhesion in vitro are usually high, in the millimolar range, because the affinity of the saccharides for the bacterial lectins is low. It can be increased several orders of magnitude by covalently linking a hydrophobic residue such as phenyl or methyl umbelliferyl to the saccharide [7]. Affinity can be similarly increased by attaching many copies of the saccharide to a suitable carrier, yielding multivalent adhesin inhibitors, as demonstrated for *Helicobacter pylori* [8,9] and for type 1 fimbriated *Escherichia coli* [10].

The feasibility of using saccharides to protect against experimental infections by bacteria expressing adhesive lectins was first demonstrated more than two decades ago [11]. Administration of methyl α-mannoside together with *E. coli* expressing the mannose-specific type 1 fimbrial lectin into the bladders of mice reduced the extent of bladder colonization by uropathogenic *E. coli* by about two-thirds compared to animals that had received the bacteria alone or with methyl α-glucoside, a sugar that does not inhibit the mannose-specific bacterial lectin. Subsequently, many studies have confirmed the ability of saccharides to prevent experimental infections caused by different pathogenic bacteria in a variety of animals (Table 1). A recent study performed in primates may be of particular significance [12]. Six rhesus monkeys naturally infected by *H. pylori* were treated with sialyl-3′-lactose [NeuAc α(2-3)Galβ(1-4)Glc], a specific inhibitor of adhesion of *H. pylori* to human gastric tissue culture cells. Two of the animals were cured of the infection and one animal cleared the infection transiently. Three other animals remained colonized. Another six animals received sialyl-3′-lactose in combination with conventional anti-ulcer drugs. Decreases in colony counts were found in three of these animals, while two animals cleared the *H. pylori* and there was no effect in the sixth. The results, albeit in small numbers of animals, suggest that sialyl-3′-lactose could be a safe and effective anti-adhesive agent for treating *H. pylori* infections.

There have been only a few clinical trials in humans

employing saccharides for anti-adhesion therapy. In one of these, 254 children were given a sialyl-3′-lacto-*N*-neotetraose [NeuAc α(2-3)Galβ(1-4)GlcNAcβ(1-3)Galβ(1-4)Glc] nose spray twice daily for 3 months to prevent otitis media. This infection is caused primarily by *Streptococcus pneumoniae* and *Haemophilus influenzae*, and to a lesser extent by *Moraxella catharralis* [13]. These bacterial species are known to produce multiple adhesins, only some of which are specific for sialyl-3′-lactose. In the clinical trial, there was no effect of the administered sugar as compared to the placebo group in preventing otitis media or colonization of the upper respiratory tract by the bacteria. It is possible, therefore, that, as suggested above, targeting only one out of several adhesins that the pathogens are capable of expressing may frequently be insufficient to prevent colonization and symptomatic infection.

In a study of 58 patients with acute otitis externa, 36 were found to be culture positive for *Pseudomonas aeruginosa* [14,15]. The patients were treated by local administration of gentamicin or a mixture of galactose, mannose and *N*-acetylneuraminic acid, sugars specific for two *P. aeruginosa* lectins, PA-I and PA-II, which may act as adhesins following their release from the bacterial cytoplasm [16]. The gentamicin-treated patients recovered more rapidly than did the patients treated with the sugars, but recovery of the latter was more rapid than that of the untreated control patients.

3. Adhesin analogs as anti-adhesive agents

The strategy for using adhesin analogs to prevent infections is based on the assumption that the isolated adhesin molecule, or an active synthetic or recombinant fragment, binds to the receptor and competitively blocks adhesion of the bacteria [1]. It has thus far been impractical to use analogs of adhesins for anti-adhesion therapy, because they are typically macromolecules that are not readily available and because they must be employed at relatively high concentrations. In addition, careful consideration must be given to their toxicity and immunogenicity. Nevertheless, modern proteomics and recombinant biotechnology have permitted the development of unique types of relatively small peptides for anti-adhesion therapy, as reported by Kelly et al. [17]. In these studies, the inhibitor employed was a synthetic 20-residue peptide mimicking the sequence of a *Streptococcus mutans* cell surface adhesin which mediates the binding of the bacteria to a salivary protein on dental surfaces. In vitro, this peptide inhibited the binding of the streptococci to the immobilized salivary receptor (i.e. an artificial tooth pellicle). Application of the peptide to teeth that had been pretreated with chlorhexidine gluconate to reduce normal microbiota and eliminate *S. mutans* significantly retarded re-colonization of the teeth with these bacteria, but had no effect on re-colonization by *Actinomyces naeuslundii*. Re-colonization by *S. mutans* was observed in control volunteers receiving saline or a control peptide. These results are promising, especially because the peptide employed was small. While conceptually encouraging, the above results must, nevertheless, be interpreted with some degree of caution, because the adhesion of *S. mutans* may also be mediated by other adhesins unrelated to the one on which this peptide sequence was based. For example, these bacteria utilize sucrose to synthesize a secreted glucan which can become immobilized on tooth surfaces. Adhesion to immobilized glucan is then mediated via a glucan binding protein expressed on the bacterial surface. A mixture of peptides corresponding to the multiple adhesins that are known to be expressed by the streptococci should prove to be more effective. The premise that peptides derived from a protein adhesin can serve as potent inhibitors of adhesion is supported by data from other experimental systems. For example, synthetic peptides mimicking a frag-

Table 1
Carbohydrates that prevent bacterial colonization and/or infection in vivo

Organism	Animal, site of infection	Inhibitor	Reference
C. jejuni	mouse intestine	milk oligosaccharides	[30]
E. coli (type 1 fimbriated)	mice UT	MeαMan	[11]
	mice GT	mannose	[79]
E. coli (P fimbriated)	mice UT	globotetraose	[80]
		P1 antigen	[81]
		(Galα1,4Gal)-containing Gp[a]	
	monkey UT	Galα1,4GalOMe	[82]
E. coli K99	calf GT	glycopeptides	[3,83]
Klebsiella pneumoniae (type 1 fimbriated)	rat UT	MeαMan	[84]
H. pylori	piglet GT	sialyl-3′-LacNAc	[12]
	monkey GT	sialyl-3′-Lac	
Shigella flexneri (type 1 fimbriated)	guinea pig eye	mannose	[85]
S. pneumoniae	rabbit lungs and rat lungs	sialyl-3′-Galβ(1-4)LacNAc	[86]
S. sobrinus	rat oral cavity	oxidized α1,6glucan	[87]
S. pyogenes	mouse pharynx	hyaluronan	[22]

[a]Gp, glycoproteins found in dove and pigeon eggwhite.

ment of the fimbrillin adhesin of *Porphyromonas gingivalis* were found to inhibit adhesion of the organisms to hydroxyapatite [18,19].

Non-proteinaceous adhesins may also be useful for anti-adhesion therapy, as shown in studies of the lipoteichoic acid (LTA)-mediated adhesion of group A and group B streptococci. In one such study, LTA was applied to the oral cavity, perineum and nape of 5-day-old mice [20]. The mothers were painted with a suspension of group B streptococci in their oral cavity and vagina or on the nipples. None of the experimental pups were culture positive after 3 days, whereas 47% of control pups were culture positive. In another study, LTA was instilled into the nasal cavities of mice, followed by administration of a group A streptococcal suspension [21]. The incidence of colonization and death of the LTA-treated mice was significantly reduced as compared to that of the control mice. Although this proves a principle, use of LTA in anti-adhesive therapy would probably not be advisable because of toxicity and/or proinflammatory properties.

Group A streptococci may bind to the CD44 receptor on epithelial cells via their hyaluronan capsule [22]. CD44 is normally a host cell receptor which binds to the hyaluronan of the host extracellular matrix to maintain the integrity of the tissue [23]. Application of hyaluronan in the oral cavity of mice prevented adhesion and colonization by group A streptococci (Wessels et al., 2001). The hyaluronan capsule of *Streptococcus pyogenes* may also act indirectly to block adhesion, by masking adhesins and blocking their access to cellular receptors by steric hindrance [24]. The capsule also acts as chelator of cations, some of which may also be required for proper function of other adhesins [25].

4. Dietary inhibitors of adhesion

Some of the most efficient anti-adhesion agents identified thus far are present in foodstuffs. Foodstuffs containing either a mixture of inhibitors or an inhibitor with a broad spectrum of activity could be especially effective. While it may be possible to find suitable inhibitors for particular pathogens, it is unlikely that it will be possible to match every individual or group of pathogens with specific diets that contain complementary adhesin inhibitors. Empirical observations over the years have suggested that certain dietary constituents may have beneficial effects on bacterial infections. These dietary constituents represent good candidates for anti-adhesion studies. However, caution should be used, because some dietary components may also be bactericidal and/or bacteriostatic and selective pressures imposed by such compounds are undesirable and should be avoided [26]. Another consideration is that adhesion may be promoted by particular dietary constituents, such as lectins, which are abundant in many foodstuffs (see below). Critical evaluation of each of these materials is, therefore, essential.

Human milk and plant-derived constituents will serve as examples to highlight the possibility of modulating adhesin activities by dietary constituents. Human milk is rich in oligosaccharides and related compounds to which many bacteria bind (Table 2) and it has been known for some time to be beneficial in preventing certain bacterial infections [27–29]. It was not until the key role of lectin–carbohydrate interactions in microbial adhesion was demonstrated that investigators began to explore the therapeutic effects of milk oligosaccharides and glycoproteins. Table 2 lists milk glycoconjugates that act as inhibitors of bacterial adhesins. In one study, it was found that fucosylated oligosaccharides in milk block infection in mice caused by *Campylobacter jejuni* [30]. Very recently, the first clinical evidence has been presented that the relative quantity of oligosaccharides in maternal milk is associated with protection of breast-fed infants against diarrhea [31]. This was based on the results of a study of a cohort of breast-feeding mother–infant pairs up to 2 years postpartum, with feeding and illness data collected weekly. In addition, the concentrations of fucosylated oligosaccharides were determined in maternal milk samples collected 1–5 weeks postpartum. It was found that the infants had a significantly

Table 2
Glycoconjugates and saccharides from human milk that interact with bacteria

Glycoconjugate	Bacterium	References
Caseinoglycopeptides	*H. pylori*	[88]
	S. sobrinus and *S. mutans*	[89]
Fucosylated saccharides	*E. coli*	[90]
Glycoprotein (40 kDa)	*S. mutans*	[91]
Glycoprotein	*S. aureus*	[92]
Galβ(1-4)GlcNAc and Galβ(1-3)GlcNAc	*P. aeruginosa*	Ramphal et al., 1991
Neutral oligosaccharides	*S. pneumoniae* and *H. influenzae*	Andersson et al., 1983, [93]
Sialylated glycoproteins	*Mycoplasma pneumoniae*	[94]
Sialyl-3′-Lac	*H. pylori*	[95]
Sialylated poly(*N*-acetyl lactosamine)	*M. pneumoniae*	[96]
Sialylated poly(*N*-acetyl lactosaminoglycans)	*S. suis*	[97]
Sialyl-3′-Lac and sialylated glycoproteins	*E. coli* (S fimbriae)	[98]
Sialylgalactosides	*E. coli* (S fimbriae)	[36]

lower risk of moderate to severe diarrhea due to *Campylobacter* spp. if the milk was rich in fucosyl-2′-lactose.

Other constituents capable of binding to pathogenic bacteria and inhibiting adhesion are present in human milk. The glycoprotein, lactoferrin, a common constituent of milk, has been shown to inhibit adhesion of *Actinobacillus actinomycetemcomitans*, *Prevotella intermedia* and *P. nigriscens* to fibroblast monolayers and reconstituted basement membranes via its protein moiety [32,33]. Lactoferrin was also found to inhibit binding of *E. coli* to HeLa cells and to red blood cells [34,35]. Secretory component, often found in human milk in considerable concentrations, inhibited adhesion of CFA-I-expressing enterotoxigenic *E. coli* to epithelial cells [35]. Mucins in human milk were shown to inhibit adhesion of S fimbriated *E. coli* to buccal epithelial cells [36].

Milk contains fat globules which can carry glycoconjugates specific for bacterial adhesins. For example, adhesion of S fimbriated *E. coli* to buccal epithelial cells was inhibited by sialylated fat globules [37]. Colostrum lipids inhibited adhesion of *H. pylori* and *Helicobacter mustelae* to immobilized glycolipids [38]. β-lactoglobulin was shown to bind to *Listeria monocytogenes* [39]. In addition to the various compounds mentioned, human milk contains antimicrobial agents such as lysozyme, lactoperoxidase and immunoglobulins [40]. A well-designed study is needed to define the individual contributions of these numerous antimicrobial and anti-adhesive components to the overall beneficial effects of human milk in preventing infections.

Because of their ready availability, plant materials possessing anti-adhesin activities are attractive candidates for antibacterial agents. There is, however, a relative paucity of information regarding the anti-adhesive properties of most plant materials (Table 3). Although plant lectins are well represented in the human diet, and many of these lectins are well characterized [26,41,42], their application to anti-adhesion therapy is very limited. Theoretically, these lectins could interact with animal cell surface saccharides to block adhesion mediated by lectin-carrying bacteria and they may enhance clearance of bacteria from the host [43].

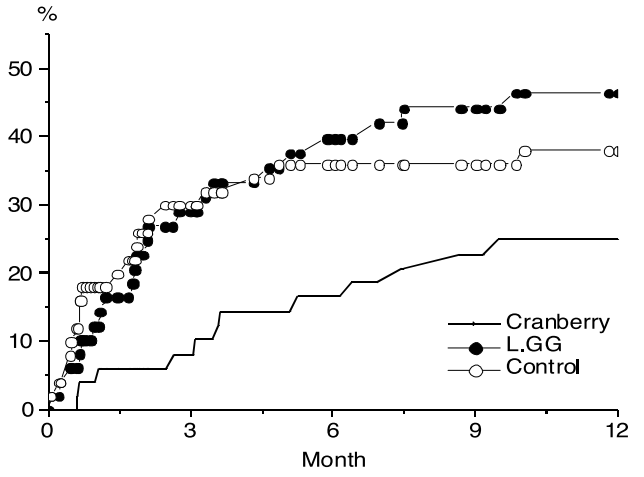

Fig. 2. Effect of cranberry juice and lactobacillus GG (probiotic) drink on first recurrence of urinary tract infection. Middle-aged women (mean age of 30) consuming 200 ml per day of cranberry juice for 6 months ($n = 50$), probiotic drink for 12 months ($n = 49$) or no intervention ($n = 50$) were followed up for 12 months. Occurrence of urinary tract infection was significantly lower in the cranberry group than in the control group ($P = 0.014$ at 6 months, 0.052 at 12 months). From [56] by permission.

Food lectins may, however, have deleterious effects as well. They may bind to mucosal cells and thus function as receptors for bacterial glycans and enhance bacterial adhesion to the tissues. For example, buccal epithelial cells scraped from individuals who had consumed raw wheat germ had high concentrations of wheat germ agglutinin on their surfaces. As a result, the cells bound increased numbers of *Streptococcus sanguis* [44]. Certain dietary lectins may reach the alimentary tract in a functional form [45] and may similarly enhance the binding of the bacteria to the different parts of the intestine.

A practical advantage in the search of dietary plant extracts for agents to use in the therapy of bacterial infections is that clinical trials may be easier to perform, mainly because toxicity is usually not as much of an issue. Among the plant extracts listed in Table 3, those obtained from cranberry (*Vaccinium macrocarpon*) are the most thoroughly studied with respect to their anti-adhesion ac-

Table 3
Anti-adhesin activity of plant constituents

Plant	Constituent	Bacterium affected	Clinical experience	References
Azadirachta indica (neem stick)	ND	*S. sanguis*	NT	[99]
Camillia sinensis (green tea)	(-) epicatechin gallate, (-) gallocatechin gallate	*P. gingivalis*	reduction of caries risk	[100]
(oolong tea)	polyphenol	*S. mutans* and *S. sobranus*	NT	[101]
Gilanthus nivalis (snowdrop)	mannose-sensitive lectin	*E. coli*	NT	[102]
Gloipeltis furcata and *Gigartina teldi* (seaweeds)	sulfated polysaccharides	*S. sobrinus*	NT	[103]
hop bract	polyphenols (36–40 kDa)	*S. mutans*	NT	[104]
Melaphis chinensis	gallotannin	*S. sanguis*	NT	[99]
Persea americana (avocado)	tannins	*S. mutans*	NT	[105]
Legume storage proteins	glycoprotein	*E. coli*	NT	[106]

ND = not determined; NT = not tried.

tivities in vitro and, thus far, are the only ones shown to be effective in clinical trials (Table 4). The initial in vitro experiments on the effects of cranberry extracts on bacterial adhesion were stimulated by the long known anecdotal evidence on the beneficial effects of cranberry juice consumption in therapy of urinary tract infections [46–48].

Several lines of evidence implicate two different cranberry constituents as active anti-adhesive agents. One of these is a high molecular mass (>15 kDa) material and the other is a protoanthocyanin. The high molecular mass material is devoid of proteins and carbohydrates and behaves in some respects like a tannin [49–51]. It inhibited the adhesion to animal cells of uropathogenic *E. coli*, including P fimbriae-, S fimbriae- and non-fimbrial adhesin I (NFA-I)-expressing strains, but did not act on the adhesins of diarrheal *E. coli* (e.g. enterotoxigenic *E. coli*), nor did it act on the type 1 fimbrial lectin [50]. In addition, it inhibited the coaggregation of Gram-negative pairs of oral bacteria more often than it inhibited coaggregations between Gram-positive bacteria [52]. Extracts containing protoanthocyanins in their condensed form inhibited adhesion of P fimbriated *E. coli* to erythrocytes [53,54].

The in vitro observations on the anti-adhesive effects of cranberry components have now been substantiated by two controlled clinical trials testing the efficacy of consumption of cranberry juice cocktail compared to a placebo that was designed to look and taste like cranberry juice. In one trial elderly women consumed 300 ml of cranberry cocktail or placebo per day for 6 months [55]. In the other study, middle-aged women drank 50 ml of cranberry–lingonberry juice (concentrated or diluted to 200 ml of water) a day also for 6 months (Fig. 2; [56]). The incidence of bacteriuria (e.g. presence of more that 10^5 bacteria per ml urine) was reduced considerably in both studies as compared to placebo. It should be recalled that cranberry juice is usually consumed as a cocktail that contains a number of additives, including 20% fructose, high concentrations of which inhibit the *E. coli* type 1 fimbrial lectin [49].

5. Sublethal concentrations of antibiotics

Subinhibitory concentrations of antibiotics have been shown to reduce the ability of pathogens to adhere to various substrates [52]. There is, however, little evidence that sublethal concentrations of antibiotics are beneficial in the therapy of infections. This apparent contradiction may be due to a number of factors, including the paucity of pilot clinical trials or well-designed in vivo animal experiments. Nevertheless, this is an important issue to study, if for no other reason that there is a high likelihood that a significant fraction of antibiotic-treated patients undergo periods in which the concentration of the drug in their system is sublethal, at least transiently, due to poor compliance.

In one study of the effects of sublethal concentrations of penicillin on urinary tract infections, 20 patients with greater than 10^5 *E. coli* per ml of urine were treated with low doses of the antibiotic [57]. Sixteen patients were said to be cured 3–7 days later, as evidenced by a reduction of urine *E. coli* to less than 10^4 per ml. None out of 18 age- and sex-matched control patients were considered to be cured. In this regard, it should be pointed out that there are several reports showing, instead, an increase in adhesion of pathogens grown at sublethal concentrations of antibiotics [2]. Interestingly, the potential anti-adhesion activity of antibiotics has become a factor of promotional consideration by at least one pharmaceutical company. In an advertisement presenting a new antibiotic (Monurol®, Rafa Laboratories, Ltd), the inhibition of bacterial adhesion to uroepithelial cells was cited [58].

6. Adhesin-based vaccines

While the beneficial effects of immunity acquired by vaccination has been known for a long time, anti-adhesion approaches emerged only after realizing the essential nature of adhesion in the infectious process and are, there-

Table 4
Anti-adhesion effects of juices or extracts of *Vaccinium* spp. (cranberry)

A. In vitro studies				
Bacterium	Disease	Adhesion assay	Effect	Reference
E. coli	UTI and pyelonephritis	HA, UroEp	I	[49,50,107,108]
E. coli	diarrhea	HA	NI	[50]
E. coli	meningitis	HA	I	[50]
Oral bacteria	dental decay periodontitis	coaggregation and buccal epithelial adhesion	I[a]	[52]
H. pylori	gastric ulcer	human gastric mucus, TC cells[b]	I	[109,111]
B. Clinical studies in humans				
Bacteria	Disease	Effect	Reference	
E. coli	UTI	reduced incidence of recurrent UTI	[55,56]	
S. mutans	dental decay	decrease in salivary *S. mutans* counts	[110][c]	

I or NI, inhibition or no inhibition of adhesion by either cranberry juice or by a high molecular mass, non-dialysable material (NDM). ND, not done; TC, tissue culture cells; HA, hemagglutination assay; UroEp, uroepithelial cells.

[a]NDM inhibited most frequently when at least one of the interacting partners was a Gram-negative bacteria.

[b]Burger et al., unpublished observations. In all assays, NDM inhibited sialic acid-specific adhesion and heparin-specific adhesion.

[c]Weiss et al. (unpublished observations).

fore, relatively new. Prevention of symptomatic infections by blocking adhesion using adhesin-based vaccines can be achieved either by active or passive means. Although active anti-adhesin immunity is expected to prevent infection by stimulation of secretory IgA on mucosal surfaces, significant amounts of serum IgG also appear to reach these surfaces, such as the gut, oral cavity and even the urinary tract [59]. In passive immunity, the target host is treated with anti-adhesin antibodies made in another host. The best example of this is where the K88 fimbriae and related adhesins of farm animal pathogens were used to vaccinate the mothers so that the suckling piglets acquired milk-secreted antibodies which functioned to prevent infection by the pathogen [60]. It has been assumed that these antibodies prevented infection by inhibiting adhesion.

Antigenic variability of protein adhesins may compromise the efficacy of anti-adhesin vaccines, especially when one considers that each of the multiple adhesins produced by a particular pathogen can undergo allelic variations, giving rise to so-called iso-adhesins (e.g. adhesins that maintain their receptor specificity, but differ antigenically). For example, there are numerous allelic variants of the FimH adhesin subunit of type 1 fimbriae of *E. coli* [61]. It has been suggested that a conserved region of an adhesin may serve as a vaccine especially if this region contains the receptor binding domain [62,63].

Although it has been known for more than three decades that anti-adhesin antibodies effectively inhibit adhesion, there are no reports on herd immunizations with purified adhesins or with recombinant strains containing the DNA encoding for the adhesin. It has been suggested that mucosal immunity, as opposed to systemic, IgG-mediated immunity, can improve the protective effect of adhesin-based vaccines [63,64]. In some cases, whole attenuated or inactivated bacteria that carry the adhesin have been employed and the anti-adhesin antibodies that were generated functioned in concert with antibodies against other virulence factors to protect the vaccinated host from symptomatic infection. For example, the inactivated *Bordetella* vaccine contains the hemagglutinin adhesin. Vaccinated individuals developed anti-hemagglutinin antibodies which were shown to inhibit adhesion of *Bordetella* to epithelial cells [65].

In animal models, active immunization with adhesins provides IgG- and/or secretory IgA-mediated protection against infection (reviewed in [66]). The choice of animal model for the study of the role of immunoglobulins in mucosal infections is of paramount importance and may be a major drawback in attempts to extrapolate the results obtained to humans [63]. Active immunization with a complex of the FimH adhesin and its periplasmic chaperone, FimC, was shown to protect against urinary tract infection caused by *E. coli* in both mice and non-human primates [62,67].

In contrast to active immunization, the passive administration of anti-adhesin antibodies may be more useful. The success of antibodies in milk in protecting against diarrheal infections in suckling piglets and calves is one example [60]. In another study, anti-*S. mutans* monoclonal antibodies directed against the bacterial SA I/II adhesins were applied onto the tooth surfaces of human volunteers that had been treated with chlorhexidine to eliminate the *S. mutans* microbiota prior to the passive application of the antibodies [68]. The re-acquisition of *S. mutans* in the placebo group receiving irrelevant monoclonal antibodies occurred within 2–3 months, whereas the passively immunized subjects remained virtually free of *S. mutans* for at least a year. However, because the anti-adhesin antibodies were no longer detectable a day after application, it is difficult to explain these findings. One very reasonable speculation is that transiently inhibiting *S. mutans* adhesion enabled other non-pathogenic oral bacteria to colonize the tooth surfaces, therefore preventing *S. mutans* from re-colonizing the occupied surfaces [69]. Nevertheless, these studies are promising and, in the future, consumption of milk from immunized cows might be especially useful in the prevention of infections in targeted populations.

7. Host-derived anti-adhesins in innate immunity

In recent years, increased attention has been given to immediate defense mechanisms based on non-clonal recognition of microbial components (i.e. innate immunity). One facet of this is the inhibition of adhesion of pathogens, and subsequent reduction of colonization by constituents in various body fluids is now known to be an important component of the innate immune system. Potential inhibitors of adhesion are abundant in body fluids, but only for a few of them is there evidence to suggest that they act to provide effective defense against pathogens. For example, the hydrophobic molecule sphinganine, a component of sphingolipids, decreases adhesion *S. mitis* to buccal epithelial cells and *Staphylococcus aureus* to nasal mucosal cells [70]. Probably the host-derived components having the greatest potential to provide innate immunity via inhibition of adhesion are those found associated with those present on mucosal surfaces as well as the secreted mucins [1]. It is assumed that some of these components act by specific binding to pathogens, trapping them within the mucus blanket, thus preventing their access to the underlying epithelial cells. In this case, reduction in colonization and infectivity would also be dependent on the flow rate of the mucus. Conditions which retard mucus flow rates may, therefore, lead to promotion of colonization [71]. Mucus may also prevent access to the underlying epithelial cells by forming a passive protective barrier [72]. In either case, it is important to identify any adhesin-reactive components in mucus in order to evaluate their potential role in innate immunity.

Mucus is a very complex mixture containing acidic poly-

saccharides, glycoproteins, glycolipids and ions [1,73]. Glycoconjugates containing oligosaccharide residues recognized by lectins expressed by the pathogens are most likely to modulate adhesion. For example, Tamm–Horsfall glycoprotein which interacts with type 1 fimbrial lectin in a mannose-specific fashion was found in urinary mucus [2,74]. In gastric mucus, a sulfated component was identified as an inhibitor of *H. pylori* adhesion to animal cells [75]. Mucus constituents may change during inflammation, resulting in an inability to inhibit adhesion [76].

The secretor or non-secretor status of individuals is of particular interest in relation to their susceptibility to infection and the possible role of soluble adhesin receptors. It has long been known that there are polymorphisms with respect to the presence (secretors) or absence (non-secretors) of blood group determinants in the fluids bathing mucosal surfaces [77]. Because most blood group determinants are glycoconjugates, some of which act as receptors for bacterial adhesins, it has been postulated that the decreased susceptibility to infections in secretors may be due to inhibition of adhesion of the pathogens by the soluble blood group determinants. This is exemplified by showing that carriage of *Neisseria meningitidis* was significantly higher among non-secretors during a school outbreak of meningitis [78]. Saliva pooled from secretors inhibited adhesion of meningococci to buccal epithelial cells as compared to saliva of non-secretors.

8. Concluding remarks

The future of anti-adhesion therapy will depend on better knowledge of the properties and specificities of bacterial adhesins and on the development of appropriate agents that block adhesion. Such agents could consist of combinations of powerful carbohydrate inhibitors targeting a variety of distinct bacterial surface lectin adhesins, or non-specific and/or broadly reactive inhibitors, such as cranberry constituents, that target multiple adhesins. Once such compounds become available, they could become drugs of choice for the management of a number of infectious diseases.

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References

[1] Ofek, I., Hasty, D.L. and Doyle, R.J. (2003) Bacterial Adhesion to Animal Cells and Tissues. American Society for Microbiology Press, Washington, DC.
[2] Ofek, I. and Doyle, R.J. (1994) Common themes in bacterial adhesion. In: Bacterial Adhesion to Cells and Tissues (Ofek, I. and Doyle, R.J., Eds.), pp. 513–562. Chapman&Hall, New York.
[3] Mouricout, M. (1991) Swine and cattle enterotoxigenic *Escherichia coli*-mediated diarrhea. Development of therapies based on inhibition of bacteria–host interactions. Eur. J. Epidemiol. 7, 588–604.
[4] Kahane, I. and Ofek, I., Eds. (1996) Toward Anti-Adhesion Therapy of Microbial Infectious Diseases. Plenum Publishers, New York.
[5] Sharon, N. and Ofek, I. (2001) Safe as mother's milk: carbohydrates as future anti adhesion drugs for microbial diseases. Glycoconjug. J. 17, 659–664.
[6] Ofek, I., Mirelman, D. and Sharon, N. (1977) Adherence of *Escherichia coli* to human mucosal cells mediated by mannose receptors. Nature 265, 623–625.
[7] Firon, N., Ashkenazi, S., Mirelman, D., Ofek, I. and Sharon, N. (1987) Aromatic alpha-glycosides of mannose are powerful inhibitors of the adherence of type 1 fimbriated *Escherichia coli* to yeast and intestinal epithelial cells. Infect. Immun. 55, 472–476.
[8] Simon, P.M., Goode, P.L., Mobasseri, A. and Zopf, D. (1997) Inhibition of *Helicobacter pylori* binding to gastrointestinal epithelial cells by sialic acid-containing oligosaccharides. Infect. Immun. 65, 750–757.
[9] Mulvey, G., Kitov, P.I., Marcato, P., Bundle, D.R. and Armstrong, G.D. (2001) Glycan mimicry as a basis for novel anti-infective drugs. Biochimie 83, 841–847.
[10] Lindhorst, T.K., Kieburg, C. and Krallmann-Wenzel, U. (1998) Inhibition of the type 1 fimbriae-mediated adhesion of *Escherichia coli* to erythrocytes by multiantennary alpha-mannosyl clusters: the effect of multivalency. Glycoconjug. J. 15, 605–613.
[11] Aronson, M., Medalia, O., Schori, L., Mirelman, D., Sharon, N. and Ofek, I. (1979) Prevention of colonization of the urinary tract of mice with *Escherichia coli* by blocking of bacterial adherence with methyl alpha-D-mannopyranoside. J. Infect. Dis. 139, 329–332.
[12] Mysore, J.V., Wigginton, T., Simon, P.M., Zopf, D., Heman-Ackah, L.M. and Dubois, A. (1999) Treatment of *Helicobacter pylori* infection in rhesus monkeys using a novel antiadhesion compound. Gastroenterology 117, 1316–1325.
[13] Ukkonen, P., Varis, K., Jernfors, M., Herva, E., Jokinen, J., Ruokokoski, E., Zopf, D. and Kilpi, T. (2000) Treatment of acute otitis media with an antiadhesive oligosaccharide: a randomised, double-blind, placebo-controlled trial. Lancet 356, 1398–1402.
[14] Steuer, M.K., Herbst, H., Beuth, J., Steuer, M., Pulverer, G. and Matthias, R. (1993) Inhibition of lectin mediated bacterial adhesion by receptor blocking carbohydrates in patients with *Pseudomonas aeruginosa* induced otitis externa: A prospective phase II study. Otorhinolaryngol. Nova 3, 1993.
[15] Beuth, J., Stoffel, B. and Pulverer, G. (1996) Inhibition of bacterial adhesion and infections by lectin blocking. Adv. Exp. Med. Biol. 408, 51–56.
[16] Wentworth, J.S., Austin, F.E., Garber, N., Gilboa-Garber, N., Paterson, C.A. and Doyle, R.J. (1991) Cytoplasmic lectins contribute to the adhesion of *Pseudomonas aeruginosa*. Biofouling 4, 99–104.
[17] Kelly, C.G., Younson, J.S., Hikmat, B.Y., Todryk, S.M., Czisch, M., Haris, P.I., Flindall, I.R., Newby, C., Mallet, A.I., Ma, J.K. and Lehner, T. (1999) A synthetic peptide adhesion epitope as a novel antimicrobial agent. Nat. Biotechnol. 17, 42–47.
[18] Lee, J.Y., Sojar, H.T., Bedi, G.S. and Genco, R.J. (1992) Synthetic peptides analogous to the fimbrillin sequence inhibit adherence of *Porphyromonas gingivalis*. Infect. Immun. 60, 1662–1670.
[19] Lee, K.K., Wong, W.Y., Sheth, H.B., Hodges, R.S., Paranchych, W. and Irvin, R.T. (1995) Use of synthetic peptides in characterization of microbial adhesins. Methods Enzymol. 253, 115–131.

[20] Cox, F. (1982) Prevention of group B streptococcal colonization with topically applied lipoteichoic acid in a maternal–newborn mouse model. Pediatr. Res. 16, 816–819.
[21] Dale, J.B., Baird, R.W., Courtney, H.S., Hasty, D.L. and Bronze, M.S. (1994) Passive protection of mice against group A streptococcal pharyngeal infection by lipoteichoic acid. J. Infect. Dis. 169, 319–323.
[22] Cywes, C., Stamenkovic, I. and Wessels, M.R. (2000) CD44 as a receptor for colonization of the pharynx by group A Streptococcus. J. Clin. Invest. 106, 995–1002.
[23] Underhill, C. (1992) CD44: the hyaluronan receptor, 1992. J. Cell Sci. 103, 293–298.
[24] Courtney, H.S., Ofek, I. and Hasty, D.L. (1997) M protein mediated adhesion of M type 24 *Streptococcus pyogenes* stimulates release of interleukin-6 by HEp-2 tissue culture cells. FEMS Microbiol. Lett. 151, 65–70.
[25] Goh, C.T., Taweechaisupapong, S., Taylor, K.G. and Doyle, R.J. (2000) Polycarboxylates inhibit the glucan-binding lectin of *Streptococcus sobrinus*. Biochim. Biophys. Acta 1523, 111–116.
[26] Cowan, M.M. (1999) Plant products as antimicrobial agents. Clin. Microbiol. Rev. 2, 564–582.
[27] Ashkenazi, S. (1994) A review of the effect of human milk fractions on the adherence of diarrheogenic *Escherichia coli* to the gut in an animal model. Isr. J. Med. Sci. 30, 335–338.
[28] Kunz, C. and Rudloff, S. (1993) Biological functions of oligosaccharides in human milk. Acta Paediatr. 82, 903–912.
[29] Newburg, D.S. (2000) Oligosaccharides in human milk and bacterial colonization. J. Pediatr. Gastroenterol. Nutr. 30 (Suppl. 2), S8–S17.
[30] Ruiz-Palacios, G.M., Cervantes, L.E., Ramos, P., Chavez-Munguia, B. and Newburg, D.S. (2003) *Campylobacter jejuni* binds intestinal H(O) antigen (Fuca1, 2Gala1, 4GlcNAc), and fucosyloligosaccharides of human milk inhibit its binding and infection. J. Biol. Chem., in press.
[31] Morrow, A.L., Ruiz-Palacios, G.M., Altaye, M., Jiang, X., Guerrero, M.L., Meizen-Derr, J.M., Farkas, T., Chaturvedi, P., Pickering, L.K. and Newburg, D.S. (2002) Human milk oligosaccharide homologs of Lewis blood group epitopes and protection against diarrhea in breastfed infants. Glycobiology 12, 648.
[32] Alugupalli, K.R. and Kalfas, S. (1995) Inhibitory effect of lactoferrin on the adhesion of *Actinobacillus actinomycetemcomitans* and *Prevotella intermedia* to fibroblasts and epithelial cells. APMIS 103, 154–160.
[33] Alugupalli, K.R. and Kalfas, S. (1997) Characterization of the lactoferrin-dependent inhibition of the adhesion of *Actinobacillus actinomycetemcomitans*, *Prevotella intermedia* and *Prevotella nigrescens* to fibroblasts and to a reconstituted basement membrane. APMIS 105, 680–688.
[34] Longhi, C., Conte, M.P., Seganti, L., Polidoro, M., Alfsen, A. and Valenti, P. (1993) Influence of lactoferrin on the entry process of *Escherichia coli* HB101 (pRI203) in HeLa cells. Med. Microbiol. Immunol. 182, 25–35.
[35] Giugliano, L.G., Ribeiro, S.T., Vainstein, M.H. and Ulhoa, C.J. (1995) Free secretory component and lactoferrin of human milk inhibit the adhesion of enterotoxigenic *Escherichia coli*. J. Med. Microbiol. 42, 3–9.
[36] Schröten, H., Plogmann, R., Hanisch, F.G., Hacker, J., Nobis-Bosch, R. and Wahn, V. (1993) Inhibition of adhesion of S-fimbriated *E. coli* to buccal epithelial cells by human skim milk is predominantly mediated by mucins and depends on the period of lactation. Acta Paediatr. 82, 6–11.
[37] Schröten, H., Lethen, A., Hanisch, F.G., Plogmann, R., Hacker, J., Nobis-Bosch, R. and Wahn, V. (1992) Inhibition of adhesion of S-fimbriated *Escherichia coli* to epithelial cells by meconium and feces of breast-fed and formula-fed newborns: mucins are the major inhibitory component. J. Pediatr. Gastroenterol. Nutr. 15, 150–158.
[38] Bitzan, M.M., Gold, B.D., Philpott, D.J., Huesca, M., Sherman, P.M., Karch, H., Lissner, R., Lingwood, C.A. and Karmali, M.A. (1998) Inhibition of *Helicobacter pylori* and *Helicobacter mustelae* binding to lipid receptors by bovine colostrum. J. Infect. Dis. 177, 955–961.
[39] al-Makhlafi, H., McGuire, J. and Daeschel, M. (1994) Influence of preadsorbed milk proteins on adhesion of *Listeria monocytogenes* to hydrophobic and hydrophilic silica surfaces. Appl. Environ. Microbiol. 60, 3560–3565.
[40] Newman, J. (1995) How breast milk protects newborns. Sci. Am. 273, 76–79.
[41] Nachbar, M.S. and Oppenheim, J.D. (1980) Lectins in the United States diet: a survey of lectins in commonly consumed foods and review of the literature. Am. J. Clin. Nutr. 33, 2338–2345.
[42] Liener, I.E. (1986) Nutritional significance of lectins in diet. In: The Lectins: Properties, Functions, and Applications in Biology and Medicine (Liener, I.E., Sharon, N. and Goldstein, I.J., Eds.), Academic Press NY, pp. 527–552.
[43] Slifkin, M. and Doyle, R.J. (1990) Lectins and their application to clinical microbiology. Clin. Microbiol. Rev. 3, 197–218.
[44] Gibbons, R.J. and Denkers, I. (1983) Association of food lectins with humanoral epithelial cells in vivo. Arch. Oral Biol. 28, 561–566.
[45] Brady, P.G., Vannier, A.M. and Banwell, J.G. (1978) Identification of dietary lectins, wheat germ agglutinin in human intestinal content. Gastroenterology 75, 236–239.
[46] Sternlieb, P. (1963) Cranberry juice in renal disease. N. Engl. J. Med. 268, 57.
[47] Moen, D.V. (1962) Observations on the effectiveness of cranberry juice in urinary infections. Wis. Med. J. 61, 282–283.
[48] Papas, P.N., Brusch, C.A. and Cresia, G.C. (1968) Cranberry juice in the treatment of urinary tract infections. Southwest. Med. 47, 17–20.
[49] Zafriri, D., Ofek, I., Adar, R., Pocino, M. and Sharon, N. (1989) Inhibitory activity of cranberry juice on adherence of type 1 and type P fimbriated *Escherichia coli* to eukaryotic cells. Antimicrob. Agents Chemother. 33, 92–98.
[50] Ofek, I., Goldhar, J., Zafriri, D., Lis, H., Adar, R. and Sharon, N. (1991) Anti-*Escherichia coli* adhesin activity of cranberry and blueberry juices. New Engl. J. Med. 324, 1599.
[51] Ofek, I., Goldhar, J. and Sharon, N. (1996) Anti-*Escherichia coli* adhesin activity of cranberry and blueberry juices. Adv. Exp. Med. Biol. 408, 179–184.
[52] Weiss, E.I., Lev-Dor, R., Kashman, Y., Goldhar, J., Sharon, N. and Ofek, I. (1998) Inhibiting interspecies coaggregation of plaque bacteria with cranberry juice constituent. J. Am. Dent. Assoc. 129, 1719–1723.
[53] Foo, L.Y., Lu, Y., Howell, A.B. and Vorsa, N. (2000) A-type proanthocyanidin trimers from cranberry that inhibit adherence of uropathogenic P-fimbriated *Escherichia coli*. J. Nat. Prod. 63, 1225–1228.
[54] Foo, L.Y., Lu, Y., Howell, A.B. and Vorsa, N. (2000) The structure of cranberry proanthocyanidins which inhibit adherence of uropathogenic P-fimbriated *Escherichia coli* in vitro. Phytochemistry 54, 173–181.
[55] Avorn, J., Monane, M., Gurwitz, J.H., Glynn, R.J., Choodnovskiy, I. and Lipsitz, L.A. (1994) Reduction of bacteriuria and pyuria after ingestion of cranberry juice. J. Am. Med. Assoc. 271, 751–754.
[56] Kontiokari, T., Sundqvist, K., Nuutinen, M., Pokka, T., Koskela, M. and Uhari, M. (2001) Randomized trial of cranberry–lingonberry juice and Lactobacillus GG drink for the prevention of urinary tract infections in women. Br. Med. J. 322, 1571.
[57] Ben-Redjeb, S., Slim, A., Horchani, A., Zmerilli, S., Boujnah, A. and Lorian, V. (1982) Treatment of urinary tract infections with ten milligrams of ampicillin per day. Antimicrob. Agents Chemother. 22, 1084–1086.
[58] Patel, S.S., Balfour, J.A. and Bryson, H.M. (1997) Fosfomycin tromethamine. A review of its antibacterial activity, pharmacokinetic properties and therapeutic efficacy as a single-dose oral treatment for acute uncomplicated lower urinary tract infections. Drugs 53, 637–656.
[59] Robbins, J.B., Schneerson, R. and Szu, S.C. (1995) Perspective: hypothesis: serum IgG antibody is sufficient to confer protection

against infectious diseases by inactivating the inoculum. J. Infect. Dis. 171, 1387–1398.
[60] Moon, H.W. and Bunn, T.O. (1993) Vaccines for preventing enterotoxigenic *Escherichia coli* infections in farm animals. Vaccine 11, 200–213.
[61] Sokurenko, E.V., Chesnokova, V., Doyle, R.J. and Hasty, D.L. (1997) Diversity of the *Escherichia coli* type 1 fimbrial lectin. Differential binding to mannosides and uroepithelial cells. J. Biol. Chem. 272, 17880–17886.
[62] Langermann, S., Palaszynski, S., Barnhart, M., Auguste, G., Pinkner, J.S., Burlein, J., Barren, P., Koenig, S., Leath, S., Jonesm, C.H. and Hultgren, S.J. (1997) Prevention of mucosal *Escherichia coli* infection by FimH-adhesin-based systemic vaccination. Science 276, 607–611.
[63] Wizemann, T.M., Adamou, J.E. and Langermann, S. (1999) Adhesins as targets for vaccine development. Emerg. Infect. Dis. 5, 395–403.
[64] Mestecky, J., Michalek, S.M., Moldoveanu, Z. and Russell, M.W. (1997) Routes of immunization and antigen delivery systems for optimal mucosal immune responses in humans. Behring Inst. Mitt. 98, 33–43.
[65] Relman, D.A., Domenighini, M., Tuomanen, E., Rappuoli, R. and Falkow, S. (1989) Protein filamentous hemagglutinin of *Bordetella pertussis*: nucleotide sequence and crucial role in adherence. Proc. Natl. Acad. Sci. USA 86, 2637–2641.
[66] Ofek, I. and Doyle, R.J. (1994) Recent developments in bacterial adhesion to animal cells. In: Bacterial Adhesion to Cells and Tissues (Ofek, I. and Doyle, R.J., Eds.), pp. 321–512. Chapman&Hall, New York.
[67] Langermann, S., Mollby, R., Burlein, J.E., Palaszynski, S.R., Auguste, C.G., DeFusco, A., Strouse, R., Schenerman, M.A., Hultgren, S.J., Pinkner, J.S., Winberg, J., Guldevall, L., Soderhall, M., Ishikawa, K., Normark, S. and Koenig, S. (2000) Vaccination with FimH adhesin protects cynomolgus monkeys from colonization and infection by uropathogenic *Escherichia coli*. J. Infect. Dis. 181, 774–778.
[68] Ma, J.K., Hikmat, B.Y., Wycoff, K., Vine, N.D., Chargelegue, D., Yu, L., Hein, M.B. and Lehner, T. (1998) Characterization of a recombinant plant monoclonal secretory antibody and preventive immunotherapy in humans. Nat. Med. 4, 601–606.
[69] Kelly, C.G. and Younson, J.S. (2000) Anti-adhesive strategies in the prevention of infectious disease at mucosal surfaces. Expert. Opin. Invest. Drugs 9, 1711–1712.
[70] Bibel, D.J., Aly, R. and Shinefield, H.R. (1992) Inhibition of microbial adherence by sphinganine. Can. J. Microbiol. 38, 983–985.
[71] Sherman, P.M. and Boedeker, E.C. (1987) Pilus-mediated interactions of the *Escherichia coli* strain RDEC-1 with mucosal glycoproteins in the small intestine of rabbits. Gastroenterology 93, 734–743.
[72] Badalament, R.A., Franklin, G.L., Page, C.M., Dasani, B.M., Wientjes, M.G. and Drago, J.R. (1992) Enhancement of bacillus Calmette–Guerin attachment to the urothelium by removal of the rabbit bladder mucin layer. J. Urol. 147, 482–485.
[73] Carlstedt, I. and Sheehan, J.K. (1984) Macromolecular properties and polymeric structure of mucus glycoproteins. In: Ciba Found Symp. Mucus and Mucosa Vol. 109, pp. 157–172. Pitman, London.
[74] Pak, J., Pu, Y., Zhang, Z.-T., Hasty, D.L. and Wu, X.-R. (2001) Tamm–Horsfall protein binds to type 1 fimbriated *Escherichia coli* and prevents *E. coli* from binding to uroplakin Ia and Ib receptors. J. Biol. Chem. 276, 9924–9930.
[75] Piotrowski, J., Slomiany, A., Murty, V.L., Fekete, Z. and Slomiany, B.L. (1991) Inhibition of *Helicobacter pylori* colonization by sulfated gastric mucin. Biochem. Int. 24, 749–756.
[76] Mack, D.R., Gaginella, T.S. and Sherman, P.M. (1992) Effect of colonic inflammation on mucin inhibition of *Escherichia coli* RDEC-1 binding in vitro. Gastroenterology 102, 1199–1211.
[77] Blackwell, C.C. (1989) Genetic susceptibility to infectious agents. Proc. R. Coll. Phys. Edinburgh 19, 129–138.
[78] Zorgani, A.A., Stewart, J., Blackwell, C.C., Elton, R.A. and Weir, D.M. (1994) Inhibitory effect of saliva from secretors and non-secretors on binding of meningococci to epithelial cells. FEMS Immunol. Med. Microbiol. 9, 135–142.
[79] Goldhar, J., Zilberberg, A. and Ofek, I. (1986) Infant mouse model of adherence and colonization of intestinal tissues by enterotoxigenic strains of *Escherichia coli* isolated from humans. Infect. Immun. 52, 205–208.
[80] Svanborg-Edén, C., Freter, R., Hagberg, L., Hull, R., Hull, S., Leffler, H. and Schoolnik, G. (1982) Inhibition of experimental ascending urinary tract infection by an epithelial cell-surface receptor analogue. Nature 298, 560–562.
[81] Johnson, J.R. and Berggren, T. (1994) Pigeon and dove eggwhite protect mice against renal infection due to P fimbriated *Escherichia coli*. Am. J. Med. Sci. 307, 335–339.
[82] Roberts, J.A., Kaack, B., Kallenius, G., Møllby, R., Winberg, J. and Svenson, S.B. (1984) Receptors for pyelonephritogenic *Escherichia coli* in primates. J. Urol. 131, 163–168.
[83] Mouricout, M., Petit, J.M., Carias, J.R. and Julien, R. (1990) Glycoprotein glycans that inhibit adhesion of *Escherichia coli* mediated by K99 fimbriae: treatment of experimental colibacillosis. Infect. Immun. 58, 98–106.
[84] Fader, R.C. and Davis, C.P. (1980) Effect of piliation on *Klebsiella pneumoniae* infection in rat bladders. Infect. Immun. 30, 554–561.
[85] Andrade, J.R.C. (1980) Role of fimbrial adhesiveness in guinea pig keratoconjunctivitis by *Shigella flexneri*. Rev. Microbiol. 11, 117–125.
[86] Idänpään-Heikkilä, I., Simon, P.M., Zopf, D., Vullo, T., Cahill, P., Sokol, K. and Tuomanen, E. (1997) Oligosaccharides interfere with the establishment and progression of experimental pneumococcal pneumonia. J. Infect. Dis. 176, 704–712.
[87] Wang, Q., Singh, S., Taylor, K.G. and Doyle, R.J. (1996) Anti-adhesins of *Streptococcus sobrinus*. Adv. Exp. Med. Biol. 408, 249–262.
[88] Stromqvist, M., Falk, P., Bergstrom, S., Hansson, L., Lonnerdal, B., Normark, S. and Hernell, O. (1995) Human milk kappa-casein and inhibition of *Helicobacter pylori* adhesion to human gastric mucosa. J. Pediatr. Gastroenterol. Nutr. 21, 288–296.
[89] Schupbach, P., Neeser, J.R., Golliard, M., Rouvet, M. and Guggenheim, B. (1996) Incorporation of caseinoglycomacropeptide and caseinophosphopeptide into the salivary pellicle inhibits adherence of mutans streptococci. J. Dent. Res. 75, 1779–1788.
[90] Cravioto, A., Tello, A., Villafan, H., Ruiz, J., del Vedovo, S. and Neeser, J.-R. (1991) Inhibition of localized adhesion of enteropathogenic *Escherichia coli* to HEp-2 cells by immunoglobulin and oligosaccharide fractions of human colostrum and breast milk. J. Infect. Dis. 163, 1247–1255.
[91] Vacca-Smith, A.M., Van Wuyckhuyse, B.C., Tabak, L.A. and Bowen, W.H. (1994) The effect of milk and casein proteins on the adherence of *Streptococcus mutans* to saliva-coated hydroxyapatite. Arch. Oral Biol. 39, 1063–1069.
[92] Mamo, W. and Froman, G. (1994) Adhesion of *Staphylococcus aureus* to bovine mammary epithelial cells induced by growth in milk whey. Microbiol. Immunol. 38, 305–308.
[93] Andersson, B., Porras, O., Hanson, L.A., Lagergard, T. and Svanborg-Edén, C. (1986) Inhibition of attachment of *Streptococcus pneumoniae* and *Haemophilus influenzae* by human milk and receptor oligosaccharides. J. Infect. Dis. 153, 232–237.
[94] Roberts, D.D., Olson, L.D., Barile, M.F., Ginsburg, V. and Krivan, H.C. (1989) Sialic acid-dependent adhesion of *Mycoplasma pneumoniae* to purified glycoproteins. J. Biol. Chem. 264, 9289–9293.
[95] Johansson, L., Johansson, P. and Miller-Podraza, H. (1999) Neu5Acalpha3Gal is part of the *Helicobacter pylori* binding epitope in polyglycosylceramides of human erythrocytes. Eur. J. Biochem. 266, 559–565.
[96] Loveless, R.W. and Feizi, T. (1989) Sialo-oligosaccharide receptors for *Mycoplasma pneumoniae* and related oligosaccharides of poly-*N*-acetyllactosamine series are polarized at the cilia and apical-microvillar domains of the ciliated cells in human bronchial epithelium. Infect. Immun. 57, 1285–1289.
[97] Liukkonen, J., Haataja, S., Tikkanen, K., Kelm, S. and Finne, J.

(1992) Identification of *N*-acetylneuraminyl alpha 2→3 poly-*N*-acetyllactosamine glycans as the receptors of sialic acid-binding *Streptococcus suis* strains. J. Biol. Chem. 267, 21105–21111.

[98] Korhonen, T.K., Valtonen, M.V., Parkkinen, J., Vaisanen-Rhen, V., Finne, J., Orskov, F., Orskov, I., Svenson, S.B. and Makela, P.H. (1985) Serotypes, hemolysin production, and receptor recognition of *Escherichia coli* strains associated with neonatal sepsis and meningitis. Infect. Immun. 48, 486–491.

[99] Wolinsky, L.E., Mania, S., Nachnani, S. and Ling, S. (1996) The inhibiting effect of aqueous *Azadirachta indica* (Neem) extract upon bacterial properties influencing in vitro plaque formation. J. Dent. Res. 75, 816–822.

[100] Sakanaka, S., Aizawa, M., Kim, M. and Yamamoto, T. (1996) Inhibitory effects of green tea polyphenols on growth and cellular adherence of an oral bacterium, *Porphyromonas gingivalis*. Biosci. Biotechnol. Biochem. 60, 745–749.

[101] Ooshima, T., Minami, T., Aono, W., Izumitani, A., Sobue, S., Fujiwara, T., Kawabata, S. and Hamada, S. (1993) Oolong tea polyphenols inhibit experimental dental caries in SPF rats infected with mutans streptococci. Caries Res. 27, 124–129.

[102] Pusztai, A., Grant, G., Spencer, R.J., Duguid, T.J., Brown, D.S., Ewen, S.W.B., Peumans, W.J., van Damme, E.J.M. and Bardocz, S. (1993) Kidney bean lectin-induced *Escherichia coli* overgrowth in the small intestine is blocked by GNA, a mannose specific lectin. J. Appl. Bacteriol. 75, 360–368.

[103] Saeki, Y. (1994) Effect of seaweed extracts on *Streptococcus sobrinus* adsorption to saliva-coated hydroxyapatite. Bull. Tokyo Dent. Coll. 35, 9–15.

[104] Tagashira, M., Uchiyama, K., Yoshimura, T., Shirota, M. and Uemitsu, N. (1997) Inhibition by hop bract polyphenols of cellular adherence and water-insoluble glucan synthesis of mutans streptococci. Biosci. Biotechnol. Biochem. 61, 332–335.

[105] Staat, R.H., Doyle, R.J., Langley, S.D. and Suddick, R.P. (1978) Modification of in vitro adherence of *Streptococcus mutans* by plant lectins. Adv. Exp. Med. Biol. 107, 639–647.

[106] Neeser, J.R., Koellreutter, B. and Wuersch, P. (1986) Oligomannoside-type glycopeptides inhibiting adhesion of *Escherichia coli* strains mediated by type 1 pili: preparation of potent inhibitors from plant glycoproteins. Infect. Immun. 52, 428–436.

[107] Sobota, A.E. (1984) Inhibition of bacterial adherence by cranberry juice: Potential use for treatment of urinary tract infection. J. Urol. 131, 1031–1036.

[108] Schmidt, D.R. and Sobota, A.E. (1988) An examination of the anti-adherence activity of cranberry juice on urinary and nonurinary bacterial isolates. Microbios 55, 173–181.

[109] Burger, O., Ofek, I., Tabak, M., Weiss, E.I., Sharon, N. and Neeman, I. (2000) A high molecular mass constituent of cranberry juice inhibits *Helicobacter pylori* adhesion to human gastric mucus. FEMS Immunol. Med. Microbiol. 29, 295–301.

[110] Weiss, E.I., Lev-Dor, R., Sharon, N. and Ofek, I. (2002) Inhibitory effect of a high-molecular-weight constituent of cranberry on adhesion of oral bacteria. Crit. Rev. Food Sci. Nutr. 42, 285–293.

[111] Burger, O., Weiss, E.I., Sharon, N., Tabak, M., Neeman, I. and Ofek, I. (2002) Inhibition of *Helicobacter pylori* adhesion to human gastric mucus by a high-molecular-weight constituent of cranberry juice. Crit. Rev. Food Sci. Nutr. 42, 279–284.