Introduction
Microbial slimes are polymers secreted by microorganisms into the surrounding environment. They are differentiated from capsules by their lack of localisation around the releasing cells and their abundant production. Although slimes are secreted into the environment by suspended planktonic cells, the quantities produced are generally higher for adhered (sessile) cells. The chemical composition of the polymers may also differ under the two conditions, even for the same bacterial species. Slime producing microorganisms are problematical in many situations, especially when they are attached to surfaces in biofilms.

Microbial slimes and biofilms
Workers in industry have long recognised the problems caused by “slimes” that develop in process machinery, in storage tanks and cooling towers and on many surfaces in contact with liquids. These slimes can also be seen in the natural environment, as “streamers” in rivers and on damp rocks, as well as under spalling stone on buildings (Figure 1). They are the result of polymeric materials produced by a wide variety of microorganisms – bacteria, fungi and algae. Slime production is associated with the occurrence of biofilms. These may be defined as structured microbial communities enclosed in a self-produced exopolymer matrix (the “slime”) and adherent to abiotic or biological surfaces. The colonisation of both biological and “inert” surfaces by microorganisms is a fundamental aspect of their pathogenesis and ecology.

Any untreated water used in an industrial process may carry microorganisms. In many industries the formation of biofilms and slimes within pipework (Figure 2), cooling systems, heat exchangers and filters (Figure 3) can cause problems. The resulting losses of efficiency due to increased frictional resistance in pipes or decrease in heat exchange capabilities can result in decreased production rates and increased costs. Sloughing and erosion of the biofilm surface results in an increase in planktonic microorganisms which may include potential human pathogens e.g. Legionella pneumophila, Cryptosporidium and Giardia spp. Examples of the detrimental effects of biofilms are listed in Table 1.

Biodeterioration
Biodeterioration may be defined as “The deterioration of materials of economic importance by microorganisms”. Biodeterioration is due to any undesirable change in the properties of a material caused by the vital activities of organisms, including microorganisms, and can be brought about by mechanical processes, where the material is damaged as a direct result of the physical activity of an organism, such as its movement or growth. Chemical assimilatory processes (perhaps the most common form of biodeterioration) occur when a material is degraded for its nutritive value, whilst chemical dissimilatory processes

Also in this issue (page 5):

Prevention of bacterial colonisation at mucosal surfaces – new technology

Julian K-C Ma and Charles G Kelly
Immunology Unit, Department of Oral Medicine and Pathology, Guy’s, King’s and St Thomas’s School of Medicine and Dentistry, King’s College London, UK.

Maturing tobacco plant, a potential production source of recombinant proteins
occur when metabolic products damage a material by causing corrosion, pigmentation or the release of toxic metabolites. Within a biofilm it is possible that all three types of biodeterioration may be in operation at the same time.

However, microbial slimes are not necessarily a problem to Man. A number of microorganisms produce large amounts of extracellular polysaccharides, which are of industrial use. Perhaps the best-known example is xanthan, produced by the bacterium *Xanthomonas campestris* (see Figure 4), which has various industrial applications, for example, as a foam stabiliser and a thickener in the food and drink industry.

### Composition of slime

Slimes are generally polysaccharidic materials, although other polymers may also be present. Their function is probably protection of the microbial cells; moreover, some of the producing organisms have shown them to be involved in the formation of biofilms on surfaces. Exopolymers (EPS) have been considered to be involved in the first steps of biofilm formation (microbial adhesion to an inert surface) and in the formation of biofilms with “normal” architecture. However, Pringent-Combaret et al. found that the *E. coli* exopolysaccharide colanic acid was involved only in the ability of the cells to produce a “normal”, voluminous biofilm, and not in the adherence of the cells to plastic surfaces, while Gaylarde & Beech demonstrated that lipopolysaccharides of the outer membrane of *Pseudomonas* spp. and sulphate-reducing bacteria were the important molecules in initial adhesion to a metal surface.

Biofilm formation in an aqueous environment occurs in the following steps:

1. Attachment of organic molecules to the surface ("conditioning")
2. Attachment of microbial cells (mainly bacteria in the first stages)
3. Replication of the sessile cells and production of EPS and other metabolites
4. Death of some cells, continued replication of others and sloughing of parts of the biofilm.

The fully mature biofilm in nature is not homogeneous. Recent studies on ‘living’ biofilms, using techniques which do not induce changes in appearance, have shown them to be heterogeneous in structure; cells and stacks of cells scattered throughout the glyocalyx (another term for EPS), with fluid-filled gaps and channels, which may connect to the bulk liquid phase ("conditioning") (Figure 5). This consortium of sessile microorganisms may exhibit differing physiological and metabolic properties from their planktonic counterparts in response to the pH, oxygen and nutrient gradients which occur within this exopolysaccharide matrix. As a result, various niches occur which may permit the co-existence, within a biofilm, of microorganisms with conflicting growth requirements e.g. both anaerobic and aerobic bacterial populations may be isolated from the same biofilm. This important fact may be overlooked by trouble-shooting investigations in industry, when it may be considered that an aerated environment such as a cooling tower contains only aerobic or facultative microorganisms. Anaerobic niches occur beneath slimes which form on the surfaces over which the water flows.

Despite the availability and common application of a range of biocides, biofilms and slimes continue to occur and cause problems in industry. Biofouling is regarded as the accumulation and growth of living organisms and their associated organic and inorganic material on a surface. This accumulation can lead to aesthetic and functional problems.

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<th>Table 1. Some of the detrimental effects of biofilms</th>
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<td>Teeth</td>
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![Figure 1. Algal and bacterial slime beneath flaked stone on an external wall.](image1)

![Figure 2. Slime from within a pipeline. (by kind permission of Mr John Gillatt, Thor UK Ltd).](image2)

![Figure 3. Slime on a screen in a paper mill. (by kind permission of Dr Pamela Simpson, Whitewater Industries).](image3)
the structure and physiology of the cells. A thick biofilm is not required to enhance the resistance of the cells and so it is obvious that this is a multi-factorial phenomenon, involving not only bioslimes but also nutrient limitation and biocides which have molecular weights in excess of 400 and which are also water soluble.

Biocide sensitivity is also affected by the actual structure of the biofilm. A “decontrolled”, homogeneous biofilm was less resistant to some toxic chemicals than the normal patchy structure. This control is related to to inter-cellular communication and the molecules which mediate it.

**Efflux pumps**

Some bacteria can reduce the intracellular accumulation of a toxic agent through the use of efflux pumps. Efflux pumps are trans-membrane complexes of a number of protein components which may include outer membrane porin molecules. These efflux systems consist of a large, inner membrane protein which is the energy-requiring efflux pump; an outer membrane protein which may be a porin molecule which has been established in *Ps. aeruginosa*, and a periplasmic ‘link’ protein which is thought to connect the two membrane complexes by spanning the periplasmic space. The outer membrane of *Ps. aeruginosa*, for example, is an effective permeability barrier against many hydrophilic compounds and the low permeability is most simply explained by the outer membrane proteins forming pores – porin channels, which prevent entry of molecules with a molecular weight greater than 350 – 400. This size restriction will exclude many compounds such as antibiotics and biocides which have molecular weights in excess of 400 and which are also water soluble.

On ships’ hulls, it results in increased drag and loss of efficiency, with the result that more fuel is used. On metallic structures, it can be responsible for metal corrosion. Biologically influenced corrosion (BIC), microbial corrosion (MC) and microbial influenced or induced corrosion (MIC), are among the terms used to describe this phenomenon. These authors report that a survey conducted in the USA in 1986 put the cost of corrosion at $167 billion with 10% of this attributable to the effects of biological attack. The use of biocides is common in attempts to avoid such economic losses.

Organisms embedded in slime are protected from the lethal effects of biocides. It was originally considered that this was simply because the biocides were unable to penetrate the thickness of the biofilm. However, other factors could also be important. Cells on surfaces have been shown to have different growth rates and nutritional requirements than planktonic cells of the same species. The mean age of the cells in the biofilm may also affect resistance, since newly formed cells (whether sessile or planktonic) show the highest sensitivity to certain inhibitors. The bioslime matrix, which apart from water, is mainly EPS, may remove the biocide. For example, biguanides and quaternary ammonium compounds may adsorb to the EPS, thus being removed from the aqueous phase in which they are active. Slimes contain enzymes released from dead and dying cells and some of these may break down or transform the biocide. Nutrient limitation and reduced growth rates resulting from the position of bacteria within a biofilm also influences the physiology of bacteria which, in turn, alters their sensitivity to biocides. It has been shown that a thick biofilm is not required to enhance the resistance of the cells and so it is obvious that this is a multi-factorial phenomenon, involving not only bioslimes but also the structure and physiology of the cells.

**Quorum sensing**

Although bacteria are uni-cellular organisms, it is now becoming apparent that under certain conditions, these organisms need to communicate and interact with each other to perform certain activities. The best known example of this is the so-called ‘quorum sensing’ behaviour, where the bacterial population senses its density and alters its gene expression accordingly. Activities influenced by quorum sensing molecules include conjugation, luminescence, virulence, swarming and the production of antibiotics and enzymes. A variety of signalling molecules is used: Gram-positive bacteria use a range of extracellular molecules such as peptide pheromones eg. amino acids and butyrolactone/butanolide metabolites, whereas Gram-negative organisms use N-acylhomoserine lactone molecules (AHLs) and VNCs. The regulation of EPS production in bacteria has recently been shown to be related to extracellular signal compounds produced by some strains and the ability of organisms to respond to other organisms’ signalling molecules may be of particular significance in the production of EPS in a heterogeneous biofilm community.

**Gene transfer and VNCs**

It is known that biofilm organisms can conjugate and exchange genetic material through the EPS matrix, although the significance of this process is still largely undetermined. In a biofilm the cells will be closer together, making transfer of plasmids more likely to occur. This could be particularly advantageous for organisms such as *Pseudomonas* spp., which possess plasmid-encoded genes. This genus is known for its ability to produce biofilms. The role of viable but non-culturable microorganisms in a biofilm cannot be estimated easily. They can include living (metabolising) but non-growing (non-dividing) variants within a population which could contribute to the bulk of the slime. They might represent a ‘terminal’ individual, which is to be ‘sacrificed’ to provide

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**Figure 4.** Colonies of the slime-producing bacterium *Xanthomonas campestris*

**Figure 5.** Recent model of biofilm structure, showing stacks of cells embedded in slime; pores between the stacks and horizontally running channels.
extracellular metabolites (quorum sensing extracellular metabolites?) to co-ordinate and regulate the activity of the community. This may seem rather altruistic for a simple bacterial cell, but who would have considered a few years ago that a bacterial population could communicate with each other and co-ordinate their activities?

When these phenomena are coupled with the ability of both Gram-positive and Gram-negative bacteria to communicate with each other in a cell-density-dependent or growth phase manner via diffusible communication molecules, it becomes obvious that individual biofilm organisms may be able to behave as members of sociable, collective communities to regulate their gene expression in order to control various physiological processes and responses, in aid of the ‘common good’. This would certainly include the production of protective slimes, perhaps by one or two members of the community more adept at this activity, under “instruction” from other members.

Taken together, the usual function of these intrinsic resistance systems may be to protect the bacteria from naturally occurring toxic agents such as those produced by other microorganisms on entry into stationary phase (secondary metabolites and antibiotics) and by plants following injury or microbial attack as well as from predators such as protozoa. On exposure of microorganisms to biocides, it is perhaps not too surprising that the organism uses whatever means are available to enable it to resist and survive these adverse conditions.

Concluding remarks

Microbial slimes, aggregations of living and dead, active and inactive, microbial cells, are not only unattractive and potentially dangerous in industry and in the environment, but are difficult to eradicate, partially because of the protective effect of the EPS. The mechanisms which govern the formation of biofilms and the genetics of EPS production are still areas of intense research. Until we are able to control various physiological processes and responses, in aid of the ‘common good’, this would certainly include the production of protective slimes, perhaps by one or two members of the community more adept at this activity, under “instruction” from other members.

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References


For further information contact:
Martin Cunningham, Oxoid Limited, Wade Road, Basingstoke, Hants RG24 8PW, England.
Tel: (0)1256 841144. Fax: (0)1256 463388. e-mail: Oxoid@oxoid.com

The Oxoid creaFAST®, H.pylori Test

With the growing worldwide prevalence of clarithromycin-resistant H. pylori, Oxoid Limited has launched creaFAST®, H. pylori Test. The test provides rapid and accurate histological identification of H. pylori in tissue sections within just 3 hours, simultaneously indicating resistance to the macrocide antibiotic, clarithromycin.

Based on Fluorescent In Situ Hybridisation (FISH), the Oxoid creaFAST®, H. pylori Test involves the binding of highly specific DNA probes to complementary sequences on ribosomal RNA. One probe is directed to H. pylori-specific rRNA and three are directed to the three rRNA sequences that confer clarithromycin resistance. Fluorescent dyes, attached to the DNA probes, enable bound rRNA to be visualised. Following a simple hybridisation procedure, detection of the probe/rRNA complex is achieved by fluorescence microscopy.

Results are available within 3 hours and are interpreted according to the colour of fluorescence observed in bacterial cells.

The procedure is quick and easy to perform. Only routine histology is required making Oxoid creaFAST®, H. pylori easy and cost effective to implement.
Prevention of bacterial colonisation at mucosal surfaces – new technology

Julian K-C Ma and Charles G Kelly

Immunology Unit, Department of Oral Medicine and Pathology, Guy’s, King’s and St Thomas’s School of Medicine and Dentistry, King’s College London, UK.

Introduction
Dental caries results from oral infection by acid-producing bacteria and is one of the most prevalent diseases on the planet. The predominant organism is *Streptococcus mutans*, a Gram-positive coccus that is commonly regarded as part of the commensal flora. Initial infection usually occurs in infants as the teeth are erupting and one of the key features of *S. mutans* is its ability to adhere to and colonise the tooth surfaces. This adhesion occurs through a saliva-derived glyco-protein matrix that coats the hydroxyapatite surface of teeth and contains receptors for *S. mutans*.

Theoretically, dental caries can be prevented by avoidance of refined sugars and oral hygiene measures. Furthermore, the introduction of fluoride, either into drinking water or toothpastes has had a significant impact on the disease in developed countries; however, caries is still a disease that affects 50% of 5 year old children and 96% of adults. Moreover, the decline in caries that was observed in the last 3 decades is not being maintained and indeed some areas are now starting to see a rise in caries incidence. In Third World countries, caries has remained a significant problem and is widely regarded as an important target for public health measures.

In this article, we shall describe two complementary approaches that have been developed to prevent dental caries and that target the adhesion phase of bacterial colonisation. Both approaches have been used in human clinical trials and illustrate the potential of anti-adhesion strategies as a means of preventing infection at an early stage. Two classes of adhesion-blocking agent have been investigated, namely anti-adhesion antibodies and adhesin analogues. These kinds of novel antimicrobial agents are likely to have broad applications in mucosal infections and their development is timely in view of increasing concern over the spread of antibiotic resistance. A limiting factor however, is the large quantities of topical reagents required for either of these approaches. In the final section, we shall describe studies involving novel plant biotechnology for production of these molecules and the exciting potential for this relatively new eukaryotic expression system.

Adhesion and colonisation
Binding of microbial cell surface adhesins to host receptor molecules is a critical early step in microbial infection and pathogenesis. Adhesion is mediated by interaction between specific adhesins on the microbial surface and receptors on host tissue. The interaction is most commonly lectin-like with protein adhesins binding to carbohydrate receptors on host tissue although adhesion by protein-protein interactions\(^1\) and by binding of microbial surface carbohydrate groups\(^2\) has also been described. Adhesion is generally regarded as a critical step in infection and several studies have demonstrated reduced adhesion \textit{in vitro} and reduced virulence \textit{in vivo} with specific adhesin-deficient microorganisms\(^3\).

There has therefore been considerable research aimed at defining the molecular basis of adhesin-receptor interactions so as to provide a rational basis for the design of adhesin-blocking reagents that can be used to prevent infection and disease. Adhesion-blocking antimicrobial agents can be divided into three main categories, namely, anti-adhesin antibodies, adhesin analogues and receptor analogues (Figure 1). Antibodies may prevent adhesion by blocking access to the site of interaction with the host receptor (adhesion epitope), while adhesin and receptor analogues may act as competitive inhibitors of adhesion.

In the case of *S. mutans*, adhesion to teeth is mediated by a cell-surface adhesin termed streptococcal antigen I/II (SA I/II) that binds to carbohydrate groups of salivary glycoproteins adsorbed onto the tooth surface\(^4\).

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**Topical application of monoclonal antibodies in the prevention of oral disease**

In early experiments, monoclonal antibodies (MAb) raised against SA I/II were applied directly to the teeth of rhesus monkeys which were fed on a human-type diet\(^5\). This treatment...
prevented colonisation with *S. mutans* and the development of dental caries. Subsequently, trials of protective MAbS were carried out in human volunteers. Two monoclonal antibodies that map to epitopes located within aa residues 816-1213 of SAI/II which contains a major adhesion determinant have been used. Over the last 15 years, we have carried out clinical trials on approximately 140 human volunteers.

In the first series of experiments, patients had three applications of antibody prior to an attempt to implant exogenous *S. mutans* by oral rinsing. Whereas the control subjects’ teeth were immediately colonised by *S. mutans* which persisted for up to three months, those receiving the *S. mutans* specific monoclonal antibody had a much lower level of infection (approximately four-fold less) which was transient and lasted only a few days. These early experiments have shown that application of the *S. mutans* specific monoclonal antibody directly to the teeth could inhibit implantation by *S. mutans* and the approach could have clinical relevance in protecting non-infected individuals (such as infants) against initial colonisation.

In a second model examining the effects of local passive immunisation, we have used one of the protective MAb (Guy’s 13) in individuals already infected with *S. mutans*. It had already been established that topical application of antibody had no effect on *S. mutans* that were already established in the mouth, so an initial pre-treatment with a topical antiseptic (chlorhexidine) was used. This reduced the oral bacterial flora and virtually eliminated oral *S. mutans*. However, the effects of chlorhexidine were short lived, as once it was stopped, *S. mutans* re-colonised the dental plaque and saliva within 1-3 days and generally returned to its original levels within three months. We have shown that if monoclonal antibody is applied immediately after stopping the antiseptic, then re-colonisation by *S. mutans* can be completely prevented. Fc-mediated effector functions of antibody are not essential for protection since F(ab–)2 fragments were as effective as the intact antibody, although there is a requirement for bivalency as Fab fragments were not protective. The effect of the antibody is highly specific, as no differences were found in the levels of other bacterial species in the mouth. Furthermore, the protection is long lasting, up to a year, even though the course of antibody treatment was only for three weeks. The long duration of protection could not be attributed to persistence of antibody since this was no longer detectable by 24 hours after application. A model was proposed in which, following clearance with chlorhexidine, any recolonising *S. mutans* is bound specifically by anti-SA I/II MAb and prevented from adhering to the tooth surface. The bacteria may also be opsonised and phagocytosed by neutrophils in the oral cavity. The ecological niche vacated by *S. mutans* is then occupied by other (non-pathogenic) plaque microorganisms that prevent recolonisation with *S. mutans* and hence provide long term protection.

Whereas the first experimental system might be a model for preventing colonisation by *S. mutans* in children and non-infected individuals, this latter system demonstrates removal of *S. mutans* from infected patients, followed by prevention of recolonisation using the monoclonal antibody. This represents the kind of approach which could be used in adults and in particular, those who have previously suffered from dental caries and might be prone to further decay.

These studies of oral infection demonstrate that long term protection against infection may be established by a relatively short application of blocking antibody. The observation that protection is independent of Fc-mediated functions is consistent with the proposal that blocking of adhesion may be a mechanism of protection. Furthermore, the protective MAbS recognised regions within the antigen associated with adherence. Competitive exclusion by other members of the oral flora, once initial adhesion of the pathogen is inhibited, may be an important factor in providing long term protection. The significance of studies involving passive immunisation with anti-adhesin antibodies is not only that such an approach may be a useful therapy but also that it provides proof of concept and justification for the development of other categories of adhesion-blocking agents. Although passive immunisation has been widely used, relatively few studies describe antibodies that target adhesion molecules specifically. In this respect, studies aimed at preventing infection with oral pathogens have been particularly informative, not least because this non-invasive form of therapy has been applied to humans.

### Synthetic adhesion epitope inhibitor in the prevention of infection by *S. mutans*

The use of a soluble form of the adhesin or of an adhesin fragment as a competitive inhibitor that blocks microbial attachment to the host receptor may also be effective in preventing infection (Figure 1c). By exploiting the specificity of the adhesin, this approach has the potential to selectively prevent infection with specific pathogens whilst having little or no effect on other members of the indigenous flora; this may contribute to protection by competitive exclusion at sites that are normally colonised. Although many pathogens possess multiple adhesins, more than one of which may mediate initial attachment, the antibody studies indicate that targeting single adhesins can be effective at least in some infections. Infection with *S. mutans* in the oral cavity has also provided a convenient model system to investigate the use of adhesin analogues in humans.

Mapping studies using a panel of synthetic peptides, identified a peptide (p1025) corresponding to residues 1025-1044 of SA I/II that inhibited *in vitro* adhesion of *S. mutans* to salivary receptor6. In a small clinical trial, p1025 was investigated as an adhesion-blocking agent in humans9. The design of the trial was essentially identical to that used for the MAb studies in that subjects were treated with chlorhexidine so as to reduce *S. mutans* to undetectable levels before repeated applications (6 times over three weeks) of the blocking agent, p1025 (in the form of an acetylated peptide amide). In addition, subjects used a mouthwash containing p1025 every day for the first two weeks after chlorhexidine treatment. Recolonisation with *S. mutans* was evident in plaque and saliva samples in control groups receiving buffer alone or a non-inhibitory peptide from day 21 onwards. By the end of the experiment (120 days), all members of the group receiving control peptide had recolonised as had three of the four receiving buffer. In contrast, none of the subjects receiving p1025 had recolonised by day 88 and only one of the four had recolonised by the end of the experiment. As with MAb, the peptide approach was specific for *S. mutans*.

The peptide, p1025, is not toxic to *S. mutans* and was detectable in the oral cavity for only 6 hours after application. Again, the long duration of protection cannot therefore be explained by continuing action of the peptide. A similar mechanism to that proposed for MAb-mediated protection may apply, namely that p1025 initially inhibits adhesion and that colonisation is subsequently prevented by...
other plaque bacteria occupying the ecological niche. This study also suggests that an adhesion inhibitor that is only partially effective in vitro may be an effective blocking agent in vivo. Maximal inhibition of SA I/II binding to salivary receptor in vitro was approximately 70% with p1025. Furthermore, there is at least one other adhesion epitope within SA I/II and genome sequencing has identified at least six further putative adhesin molecules. Thus, in a competitive environment it may not be necessary to completely inhibit microbial attachment for the balance to be altered towards non-infection.

Other applications
The studies described have not been performed in isolation. There are many other examples of adhesion-blocking strategies used in animal models. For example, various studies using rodent models of vaginal candidiasis have been performed. Murine MAbs directed against mannan adhesins or against secreted aspartyl proteases (Saps) that may also function as adhesins, induced significant reductions in fungal burden.

The effectiveness of anti-adhesin antibodies has also been demonstrated in a site that is not normally colonised. In a murine cystitis model, passive immunisation with antiserum raised against the pilus-associated adhesin FimH produced a 100- to 150-fold reduction in the number of uropathogenic Escherichia coli recovered from the bladder (2 days after challenge) compared with serum from mice immunised with adjuvant alone.

There is one further example of an adhesion-blocking MAb used to prevent infection in a human recolonisation study. Patients with periodontitis were treated with a MAb raised against an adhesin of the putative periodontal pathogen, Porphyromonas gingivalis. Following four applications of MAb subgingivally over a period of 10 days, subjects were significantly protected against recolonisation over a period of 9 months.

The use of a synthetic peptide adhesin analogue has been investigated in a murine model of rotavirus infection. A synthetic peptide corresponding to a conserved sequence from the viral attachment protein VP4, was administered orally to synthetic peptide corresponding to a conserved sequence from the viral attachment protein VP4, was administered orally to xenogeneic receptor constructs, and was found to inhibit virus attachment.

Transgenic plants, a production system for topical therapeutic reagents
Peptides can be synthesised relatively cheaply, although, as discussed below, the market requirements for an anti-caries product is potentially enormous. On the other hand, monoclonal antibodies are much harder to produce. Moreover, in the case of S. mutans, the requirement for full length immunoglobulin (rather than antibody fragments) excludes the use of recombinant E. coli. This leaves less established expression systems as the only alternatives, which are costly, and each has its own disadvantages.

A further significant factor is the need for enormous quantities of monoclonal antibody. Unlike active immunisation with antigen, passively applied antibodies that are washed away, swallowed or degraded need to be applied repeatedly and in relatively high concentrations. It is likely that the current treatment regime will be refined, but currently a single course of antibody treatment uses approximately 22.5mg antibody. In the UK, if this treatment was provided only to children on a yearly basis, several hundred kg antibody would be required. Global requirements would extend to thousands of kg/yr, which is far in excess of the capacity of any antibody production facility currently in use.

Plant biotechnology is a new and rapidly expanding area and in the last five to ten years it has become apparent that genetically modified plant systems may be particularly valuable for the expression and production of recombinant molecules (Figure 2). A considerable effort has focused on genetic engineering for the improvement of plant characteristics and agricultural properties, but the technology has also been applied to the production of “high value” products, namely pharmaceutical compounds and vaccines. A particular attraction of this approach is the potential for growing immunotherapeutic reagents on an agricultural scale, thereby significantly reducing the costs of production. Plants are the most cost effective and energy efficient system for production of proteins on the planet, and have simple nutritional requirements, amounting essentially to sunlight, soil and water.

In 1989, the use of genetically modified plants for the expression of a full length IgG monoclonal antibody was described for the first time. It was originally a source of some surprise that these complicated molecules could be assembled by plants. Plants do not, of course, normally produce antibodies or related proteins. Antibodies are complicated molecules that have precise assembly requirements in order to produce a functional protein. We have shown that functionally, there is no discernible difference between antibody expressed in plants and that expressed by mammalian cells in culture. We have also shown that as higher eukaryotes, plant cells process the foreign antibody proteins in much the same way as mammalian plasma cells and that the cell machinery within the endomembrane system for protein folding, assembly and secretion is similar in plants and animals. This explains in part,
the fidelity and efficiency of antibody production in plants, and has implications for the prospects of producing other vaccines in plants.

We have exploited further the ability of plants to produce immunoglobulin, to engineer a secretory antibody version of Guy’s 13. Secretory antibodies are the predominant naturally occurring form of immunoglobulin found in all human secretions, including those of the mouth, eyes, lungs and gastro-intestinal tract. They are structurally considerably more complex than IgG antibodies and may have advantages over IgG because of increased avidity through dimerisation by J chain and resistance to proteolysis in the mucosal environment through association with secretory component.

The production of Guy’s 13 secretory antibody in plants was highly efficient, with levels of antibody representing 5-8% of total soluble plant protein\textsuperscript{17}. In a human trial, the plant-derived secretory antibody proved to be as effective as the parent murine IgG antibody in preventing recolonisation with \textit{S. mutans}\textsuperscript{18}. Persistence of the secretory antibody in the oral cavity was increased compared with IgG (three days compared with one day). In this infection model, although treatment with IgG is extremely effective, the increased survival time of the SIgA antibody may allow longer intervals between applications. Furthermore, in view of the increased functional affinity of SIgA over IgG, it may be possible to reduce the amount of antibody required per course of treatment. Both of these factors may be of particular importance in the prevention or treatment of infections at other, less accessible, mucosal sites.

Although peptides are relatively cheap to synthesise, a limiting factor in production could be the sheer volume of peptide required. In the only clinical trial reported so far, approximately 120mg peptide was used per course of treatment. If peptide production requirements were to run into the 1000-10,000 kg/yr range, then alternative production strategies would need to be considered. Once again, plants may offer a cost-effective solution. In this case, a second developing plant biotechnology – plant viral vectors – may be appropriate. It has already been demonstrated that peptides up to 35 amino acid residues may be incorporated into the coat protein of various plant viruses, without affecting the ability of the virus to infect, replicate or spread systemically throughout the plant\textsuperscript{19}. The recombinant virus is used to infect (non-genetically modified) plants which are essentially used as culture vessels. This system allows for very rapid production of large quantities of recombinant virus, from which the target peptide can be purified. The approach has already been used successfully to express an epitope from mink enteritis virus. Systemic immunisation with the plant-derived chimaeric viral particles conferred protection against disease in experimental animals\textsuperscript{20}.

Conclusions

Adhesion-blocking strategies can be effective in preventing microbial infection in animal models of disease and, importantly, in human models of infection. Although most bacterial or fungal pathogens possess multiple adhesins, the studies reviewed here demonstrate that targeting a single specific adhesin-receptor interaction can be sufficient to prevent infection. Turnover of mucous and epithelial cells, competition with indigenous flora, peristalsis and fluid flow present formidable barriers to the establishment of a pathogen at a mucosal site. It is therefore perhaps not surprising that addition of one further obstacle in the form of a specific inhibitor of adhesion can be sufficient to promote clearance of at least some infectious agents.

Passive immunisation with anti-adhesin antibodies can be an effective means of reducing or preventing infection when given prophylactically. In these “proof of principle” studies, antibody administration regimens were generally not optimised. It also remains to be established whether passive immunisation can be used therapeutically for infections at mucosal surfaces. Furthermore, while anti-adhesin antibodies may be effective against bacterial or fungal pathogens, it is less clear whether they will be of use against viruses where there is considerably more sequence variation. For these infections, use of adhesin or receptor analogues, may be more appropriate.

An enormous increase in the already large number of characterised microbial adhesins can be anticipated as more genome sequences become available. In turn, many more adhesion-inhibiting agents may be identified either as a result of detailed molecular analyses of adhesin-receptor interactions or from screening of combinatorial libraries or, in the case of MAbs, by use of synthetic antibody library technology. For adhesion-blocking antibodies, SIgA may well be the preferred isotype at mucosal sites, since the increased functional affinity and persistence will be advantageous.

The two approaches of adhesion blocking MAbs and adhesin analogue peptide are not mutually exclusive, rather there may well be a synergistic effect if combinations of inhibitor were to be used, to develop a more complete blockade at the potential site of infection.

Both strategies however, require the availability of large amounts of reagent and until now, this hurdle has blocked the development of these anti-microbial approaches. Developments in plant biotechnology over the last decade may provide an answer, providing that the media driven furore over genetically modified foods does not overwhelm the debate. This technology provides a means for production of antibodies, proteins and peptides, on an agricultural scale. In the case of antibodies, it also provides an effective means of engineering the isotype of full length antibodies, and is currently the only practical system for expression of secretory IgA.

References