Clin. Pharmacokinet. 22 (1): 1-10, 1992
0312-5963/92/0001-0001/$05.00/0

CPK1 109

Oral Delivery of Vaccines
Formulation and Clinical Pharmacokinetic Considerations

Derek T. O'Hagan
Department of Pharmaceutical Sciences, University of Nottingham, Nottingham, England

Oral immunisation has a long established tradition in preventive medicine; indeed, the first attempt to induce immunity in humans, more than 2000 years ago, was undertaken by the oral route (Witebsky 1967). The first book on oral immunisation was published more than 60 years ago (Besredka 1927) and the oral polio vaccine has been licensed for use in humans since 1960.

Nevertheless, the oral route of immunisation remains clinically underexploited. This is partly because it is only relatively recently that the important protective functions of secretory immunoglobulin A (sIgA) at mucosal sites has become appreciated (Mestecky & McGhee 1987). For example, a recent paper has described the passive transfer of polymeric IgA to confer local immunity against intranasal infection with influenza virus in mice (Renegar & Small 1991). Before the 1960s, it was widely believed that serum antibody 'spilled over' into the external secretions to provide mucosal immunity. However, it is now generally acknowledged that sIgA is locally produced in response to mucosal stimulation and that the mechanisms for induction, regulation and transport of sIgA are distinct from those involved in systemic immunity. Furthermore, accumulated experimental evidence supports the existence of 'a common mucosal immune system', which is linked by emigrating antigen-stimulated IgA precursor cells (fig. 1). These IgA-committed cells are stimulated by antigens absorbed through M cells, which are specialised 'antigen-sampling' cells found in the epithelium of the gut-associated lymphoid tissues (GALT) such as the Peyer's patches (Owen & Ermak 1990). The antigen-stimulated IgA-committed lymphoblasts migrate via the lymphatics into the circulation and subsequently specifically localise in mucosal tissues and secrete IgA locally (Mestecky & McGhee 1987). The most convincing evidence for the existence of the common mucosal immune system comes from studies in humans and experimental animals demonstrating the induction of mucosal immunity at distant mucosal sites such as tears, saliva and genital secretions following oral immunisation (Bergmann & Waldman 1988).

Traditionally, vaccine research has been mainly concerned with the induction of systemic immunity by parenteral immunisation. For diseases in which the infectious agent is introduced parenterally, such as tetanus and malaria, systemic immunity is clearly most appropriate. However, it is well known that most infections are acquired naturally through mucosal routes, either nasally, orally or (less frequently) genitally. Parenteral vaccines are poor inducers of mucosal immunity, a fact perhaps best illustrated by the limited efficacy of the parenteral cholera vaccine (Holmgren et al. 1989).

When compared with parenteral vaccines, oral vaccines potentially offer the advantage of greater efficacy against mucosally acquired infection because of their ability to induce an sIgA response. Furthermore, they may be safer, easier to admin-

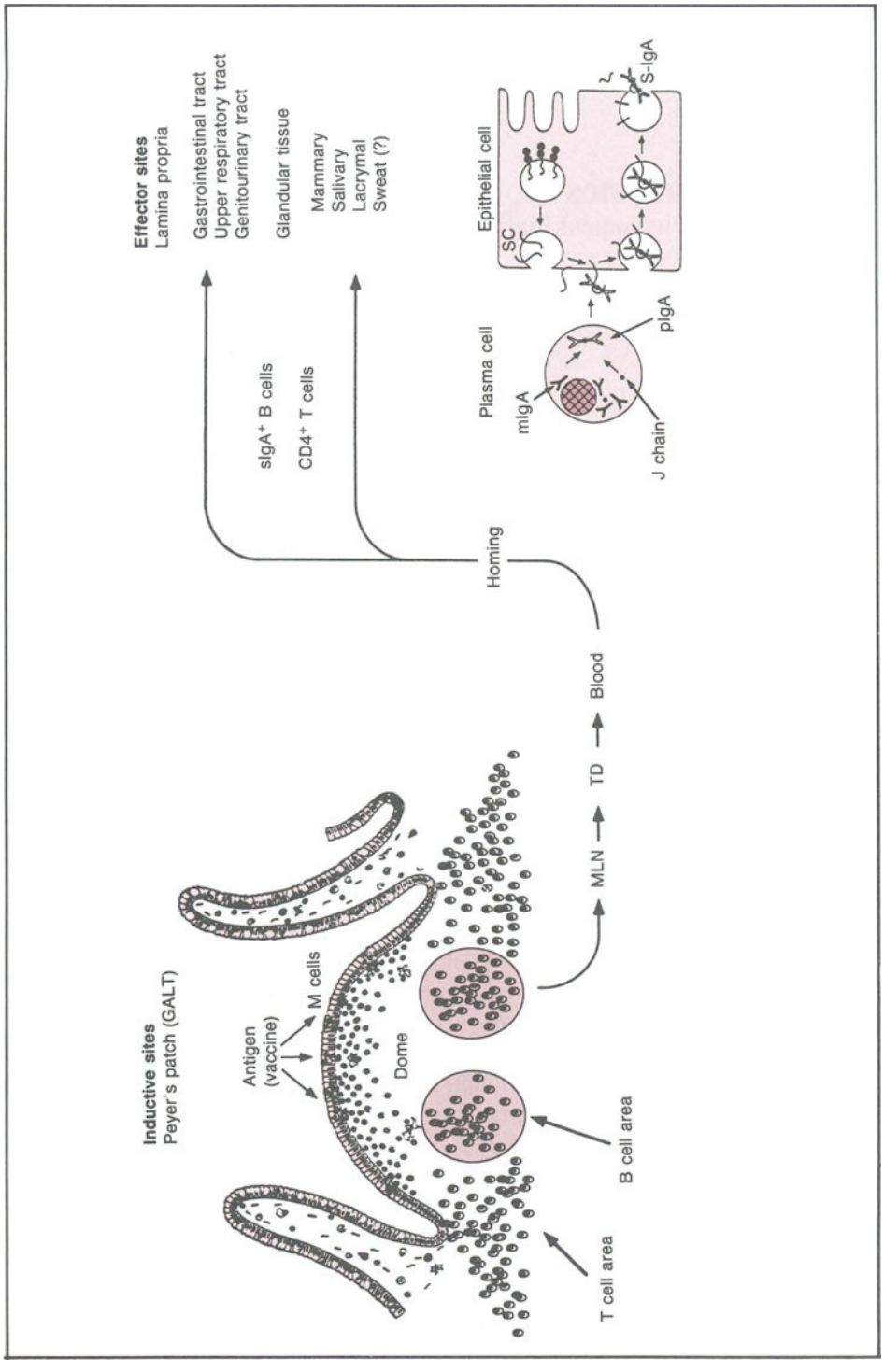

Fig. 1. Diagrammatic representation of oral immunisation-induced secretory immunoglobulin A (sIgA)-mediated responses in external secretions (effector sites). The uptake of antigen by M cells initiates the response in the gut-associated lymphoid tissue (GALT) such as Peyer's patches. Immunoglobulin A committed B cells and $CD4^+$ T helper cells sensitised to the antigen then migrate via efferent lymphatics through mesenteric lymph nodes (MLN) to the thoracic duct (TD) and thence into the circulating blood. These cells enter sites which affect the production and transport of IgA, which, with J chain – which is also produced by plasma cells and links monomeric IgA to form polymeric IgA – interacts with secretory component (SC) on the epithelial cell, is endocytosed, transported across the cell from basolateral to apical sides and is released (McGhee & Mestecky 1991).

ister and better tolerated, and have the potential for almost unlimited frequency of boosting. Moreover, since oral vaccines would not require the same level of 'purity' and quality control as parenteral vaccines, they would also be less expensive to manufacture. It is clear that oral vaccines would be particularly advantageous in the Third World, where it is often difficult to get access to the population to be immunised. Many currently available vaccines would be improved tremendously if they could be administered orally and, in addition, developments in oral vaccines could make available new vaccines against diseases which are currently poorly controlled such as infections caused by *Escherichia coli, Shigella* spp., *Vibrio cholerae* and *Salmonella* spp. Nevertheless, despite considerable advances in the basic understanding of the structural tissues, cell traffic and control of secretory immunity in recent years, success with local immunisation strategies has proved elusive. Degradation of purified antigens, poor absorption, interaction with nonspecific host factors, inadequate delivery systems and pre-existing immunity have all contrived to influence negatively the outcome of oral immunisation. Hence, the general perception is that large amounts of antigen are needed for oral immunisation and poor immune responses are often the result.

However, recent research has resulted in the development of several novel 'antigen delivery' systems which can be very efficient at inducing both secretory and systemic immunity following oral administration. These improved systems offer considerable promise for the future and may result in the development of new oral vaccines for clinical use. It is with these recent developments in antigen delivery systems that this article is concerned.

Current strategies for oral vaccine development can be divided into 2 overall areas, involving replicating (live) and nonreplicating antigen delivery systems. The first area involves a range of viral and bacterial vectors which have been genetically engineered often to express heterologous antigens from alternative pathogenic microorganisms. The second area, nonreplicating antigen delivery systems, involves the formulation of the antigens into carrier systems capable of protecting them against degradation in the gut and delivering them to the GALT.

1. Replicating Antigen Delivery Systems

In general, replicating vaccines are likely to be more effective at stimulating immunity following oral administration than nonreplicating vaccines. This is due to their ability to proliferate in host tissues after immunisation. Replication of the carrier will result in more effective presentation of antigens in conjunction with the histocompatibility (MHC) antigens of the host. In addition, since live viruses can be processed through the 'endogenous' pathway of antigen presentation, they are sometimes able to induce cytotoxic T lymphocyte (CTL) responses (Bolognesi 1990). Antigens from nonreplicating vaccines are internalised through the endosomal or 'exogenous' pathway and consequently are thought to be unable to induce CTL responses.

1.1 *Salmonella*-Based Vaccines

In recent years, considerable efforts have been directed towards the development of an effective oral vaccine against the causative agent of typhoid fever, *Salmonella typhi,* since the whole cell parenteral vaccine is associated with common and often severe side effects (Hackett 1990). The salmonellae are species which can colonise the GALT, but can also survive and persist in the host before inducing a disseminated infection of the mononuclear phagocyte system, namely the spleen, liver, lymph nodes and bone marrow. In the 1970s, an avirulent mutant strain of *S. typhi* (Ty21a) was developed using chemical mutagenesis. Subsequent field trials in humans demonstrated that the vaccine was safe and immunogenic, although protection against typhoid was moderate and unduly sensitive to vaccine formulation and dose regimen (Hackett 1990). Therefore, Ty21a is probably overattenuated and has limited immunogenicity, but no replacement is yet obvious.

However, the major problem with Ty21a is that the molecular basis of its attenuation is not under-

stood. In this age of recombinant DNA technology in which it is possible to construct strains of a well defined nature, it may be inappropriate to use Ty21a when the basis of attenuation is not understood. Nevertheless, this vaccine is available for administration to humans in over 40 countries and has been approved for use by the Food and Drug Administration (FDA) of the US and is available in the USA as 'Vivotif'. It is recommended for tourists and business travellers to endemic areas and is claimed to confer a protection rate of around 70% for up to 6 years following a single course of 4 daily doses of the vaccine. A recently reported study with Ty21a has demonstrated for the first time that an antigen-specific immune response to an orally administered live bacterial vaccine can be induced in the lower respiratory tract of humans (Forrest et al. 1991. Hence, oral delivery systems may function as vectors for the delivery of antigens from respiratory pathogens.

Second generation typhoid vaccines have now been developed by recombinant DNA techniques involving the deletion of 1 or more genes required for full pathogenicity (Chatfield et al. 1989). For example, double *aroA* and *purA* mutant strains of *S. typhi* have been constructed and administered to humans after encouraging data were generated with similar mutant strains of the closely related mouse pathogen *S. typhimurium*. In humans, the genetically modified vaccines were shown to be safe but nonimmunogenic due to over-attenuation (Levine et al. 1987). This and other studies highlighted a major experimental problem: the behaviour of *S. typhimurium* mutants in mice was not necessarily predictive of the behaviour of supposedly analogous *S. typhi* strains in humans. The development of rationally attenuated strains of salmonellae for use as oral vaccines awaits a fuller understanding of salmonella pathogenesis. For *S. typhi*, this may not be easily defined using animal models. An important recent development is the establishment of a human monocyte-macrophage cell culture system for assessing the virulence of live oral typhoid vaccines (Hackett 1990).

In anticipation of the construction of safe and immunogenic oral salmonella vaccines, the possibility of using such organisms as carriers of heterologous antigens has also been addressed (Clements 1987). A number of genetically defined attenuated mutants of salmonella have been produced by deletions in genes (*pab, pur, galE, aroA, asd, Ts, cya* and *crp*) required for full pathogenicity. Avirulent strains have resulted which can be modified to express colonisation and virulence antigens from alternative pathogens (Curtiss et al. 1989). For example, live attenuated *S. typhimurium* (*aroA* mutant) expressing the fragment C of tetanus toxin has been used to induce protective immunity against a lethal toxin challenge following oral immunisation (Fairweather et al. 1990). In addition, a typhoid-cholera hybrid vaccine developed from Ty21a has been assessed in humans (Tacket et al. 1990). The hybrid vaccine did not provide significant protection against experimental cholera, but did significantly reduce the severity of clinical illness.

In conclusion, attempts to develop rationally attenuated strains of salmonella for use as oral vaccines are progressing. In addition to providing new *S. typhi* vaccines, these strains may also function as effective carriers for foreign genes from alternative pathogens and may result in important advances in oral vaccination. Nevertheless, substantial problems remain unresolved. First, can the same carrier be used repeatedly to deliver different heterologous antigens? It seems likely that preexisting immunity to the carrier organism will limit replication in the host, resulting in reduced efficacy on repeated administration. Hence, the vaccine may be capable of being used successfully only once. Similar problems are also likely to occur with alternative live carriers, especially if they are used in endemic areas or in hosts who have been previously immunised against the carrier organism. These problems may seriously limit the use of live recombinant carriers in many areas and in many populations.

The second problem with recombinant organisms is more philosophical and relates to the safety or desirability of using genetically modified live vectors as vaccines. The potential use of live bacterial and viral vectors for vaccines was considered

Table I. Safety requirements for live vectors

Low incidence of side effects
Safe to administer to immunodeficient individuals
Infectious only for target population
Vector is not tumorigenic
Vaccines contacts cannot be infected (vector is not contagious)
No integration of vector DNA into host cell genome
No persistent infection established
Vector does not induce autoimmune disease

at a meeting convened by the World Health Organisation in 1989. The group of scientists at this meeting recommended that further vaccine research in this area should be encouraged, but stressed that safety and efficacy should be closely monitored during vaccine trials, particularly in those that may involve immunodeficient individuals (WHO 1990). The safety requirements that will have to be satisfied by any live vector prior to use as a vaccine are highlighted in table I.

However, safety and acceptability are controversial and emotive issues in many parts of the world and future policies will not be decided by scientists alone. My own personal view is that while deletion of the genes responsible for the pathogenicity of bacteria or viruses using recombinant DNA technology seems a logical and progressive step, the insertion of genes responsible for pathogenicity in 1 organism into a second carrier organism gives cause for more legitimate concern. While it is clear that safety is the major concern of both manufacturers and regulatory authorities alike, the 'true' safety profiles of new products do not become available until they are administered to large populations of humans.

The general problems and concerns associated with the release of genetically engineered microorganisms into the environment have recently been discussed (Sussman et al. 1989). It should be noted, that the promise of human benefit from recombinant DNA technology emerged most clearly in the field of vaccine development. Indeed, it is likely that new vaccines resulting from this technology will be far safer than the older vaccines, which were produced by often ill-defined attenuation techniques. It is apparent from the recommendations of the recent WHO meeting on the potential use of live vectors as vaccines, that recombinant vaccines will need to satisfy far more stringent safety criteria than traditional vaccines; clearly this is rightly so.

Attempts have also been made to construct attenuated strains of *Shigella flexneri* for use as oral vaccines (Lindberg 1988) and one such strain is currently being assessed in clinical trials. Additional research is also under way to construct shigella vaccines based on hybrid constructs of *E. coli* and *S. typhi* (Hale & Formal 1989).

1.2 Alternative Live Vectors

Alternative live vectors currently being investigated as possible oral vaccines include poxviruses, adenoviruses, polioviruses and mycobacterium bacille Calmette-Guérin (BCG). BCG, which is the most widely used vaccine in the world, is perhaps the most promising of these alternative vectors. It is very safe, can be given to the very young, induces long lasting immunity and is inexpensive to produce. Several foreign antigens have been expressed in BCG and have induced immune responses in experimental animals (Stover et al. 1991). Considerable success has been achieved in studies involving immunisation of animals with recombinant vaccines. For example, a vaccinia-rabies recombinant virus has been successfully used to orally immunise foxes against rabies by distribution of vaccine-baited food in their feeding area (Brochier et al. 1990). In addition, vaccinia virus recombinants expressing human immunodeficiency virus (HIV) antigens have been used to immunise humans in phase I clinical trials at several centres in the US.

An alternative approach is the use of adenoviruses as carrier systems for heterologous antigens. Adenovirus vaccine has been orally administered to several million US soldiers and has been shown to offer excellent protection against acute respiratory disease. Recombinant adenoviruses based on the vaccine strains have been constructed and in studies in chimpanzees and dogs (Chengalvala et

al. 1991) have been shown to be capable of inducing immune responses to the expressed antigen (hepatitis B surface antigen) following oral immunisation. Another live attenuated organism under consideration for development as a possible recombinant vaccine for humans is poliovirus. The Sabin type 1 vaccine strain of poliovirus, which is probably the safest and most successful attenuated human vaccine, has been adapted to express antigens from alternative pathogens (Burke et al. 1988). However, the ability of this poliovirus chimaera to induce antibodies following oral administration remains to be assessed and the effect of pre-existing immunity needs to be determined.

2. Nonreplicating Antigen Delivery Systems

The major advantage of nonreplicating antigen delivery systems is safety, which is of paramount importance, particularly since vaccines are mainly administered to the very young. Disseminated infection is a potential problem with all live vaccines, especially if they are used in immunosuppressed individuals. HIV-induced immunosuppression appears to be responsible for increased mycobacterial infections in a number of populations and a recent report described the appearance of disseminated BCG infection in a patient with acquired immunodeficiency syndrome (AIDS) 30 years after BCG vaccination (Ambruster et al. 1990).

Current regulatory requirements for new vaccines are much more stringent than they have been previously and several widely available 'traditional' vaccines might not have found approval if they had been newly developed today. For example, despite the apparent safety of oral polio vaccines over many years, the Sabin type 3 strain has been shown to readily revert to the virulent genotype in the gut (Evans et al. 1985) and recombination between different serotypes has been observed in healthy vaccine recipients (Minor et al. 1986). Hence, the safety of this vaccine in use is difficult to explain and if the currently available information had been available in the 1960s it is doubtful if this vaccine would ever have been approved for human use.

The efficacy of nonreplicating oral vaccines relies heavily on the ability of the vaccine formulations, or antigen delivery systems, to deliver the antigens to the GALT in an intact and immunogenic form. Hence, efficacy is heavily dependent on the design and formulation of the delivery system. Much of the research on nonreplicating antigen delivery systems as oral vaccines is very much in its infancy and few delivery systems have advanced further than experimental studies in animal models. Nevertheless, I will highlight some of the successes achieved to date and comment on the potential drawbacks of several of the systems that have been assessed. Nonreplicating antigen delivery systems for oral, nasal and parenteral immunisation have been discussed recently (O'Hagan 1990a).

2.1 Cholera Toxin and Its Subunits

As a result of their limited efficacy and acceptability, parenteral cholera vaccines are no longer regarded as clinically useful. Instead, attention has turned to the development of oral vaccines, which should be able to stimulate intestinal immunity more effectively. Two new oral vaccines have been evaluated in large scale field trials in cholera endemic areas. The vaccines consisted of either killed whole cells (WC) alone or WC in association with the purified B subunit of cholera toxin (CTB). The CTB is nontoxic and is an exceptionally potent oral immunogen, due to its ability to bind to specific receptors on the intestinal mucosa. In the trials, the WC-CTB vaccine had greater efficacy than the WC vaccine for the initial 4 to 6 months (85% protection against disease with WC-CTB, compared with 58% with WC alone). Thereafter, the level of protection was similar for the 2 vaccines, at about 60% for the 3-year period analysed (Holmgren & Svennerholm 1990; Holmgren et al. 1989). Although the level of protection was substantially reduced after 6 months in younger subjects, the parenteral vaccine offers no protection at all to many young children. Furthermore, the oral WC-CTB vaccine

Table II. Advantages of microparticles for oral immunisation

Microparticles are taken up into the gut-associated lymphoid tissue
Entrapped antigens are protected from degradation in the gut
Several antigens may be entrapped and delivered simultaneously
Adjuvants may also be entrapped and delivered simultaneously
Polymers are nonimmunogenic, so the microparticles can be used for repeated boosting
Controlled or 'pulsed' release of antigens from microparticles is possible
It should be possible to target microparticles to promote uptake

also induced significant cross-protection against enterotoxigenic *Escherichia coli* (ETEC), resulting in a 50% reduction in the overall incidence of diarrhoeal disease in the population studied. Similar WC-CTB vaccines are also being assessed for protection against ETEC and initial results are encouraging (Holmgren & Svennerholm 1990).

Recombinant DNA technology is also being used in attempts to develop a live oral cholera vaccine and *V. cholerae* strains are being developed in which the genes coding for the toxin have been deleted. However, in preliminary studies, these recombinant vaccines have induced an unacceptable level of diarrhoea in immunised individuals.

It has been shown that whole cholera toxin (CT) is a potent adjuvant for oral immunisation against unrelated antigens (Lycke & Holmgren 1986). Consequently, there has been considerable interest in the possible use of CT or CTB as adjuvants in a wide range of oral vaccines. However, since CT and CTB themselves are very potent immunogens, pre-existing immunity is likely to limit efficacy on repeated administration. The mucosal adjuvant activity of CT is thought to be due to a synergistic effect involving both the CTB and the adenylate cyclase activity of the A subunit (Wilson et al. 1990) and it has been shown that purified CTB alone is unable to act as an adjuvant (Lycke & Holmgren 1987). Thus, many of the reports describing the adjuvant effect of CTB alone may actually be due to the presence of small amounts of whole CT. In addition, if CTB or CT were to be used as oral adjuvants, it is likely that they would need to be specifically conjugated to the vaccine antigens. Otherwise, immune responses to unrelated 'bystander' antigens in the gut may occur, resulting in potentially very damaging consequences. The effect of chemical conjugation to the antigens on the binding characteristics and efficacy of CT or CTB would need to be determined and it might not be easy both to couple to antigens and to retain full adjuvant activity. In addition, the mechanism of the adjuvant activity of CT remains poorly understood, but is thought to involve effects on regulatory T cells. Hence, the acceptability of whole CT as a general vaccine component seems limited, in view of its undoubted toxicity and its potential for adverse effects.

2.2 Microparticles

The ability of microparticles to act as oral antigen delivery systems is dependent on the reported uptake of particles into the Peyer's patches of the GALT (O'Hagan 1990b). After uptake, microparticles are phagocytosed by macrophages and can subsequently be identified in the mesenteric lymph nodes, livers and spleens of experimental animals. Several recent reports have described the induction of secretory immune responses to antigens entrapped in microparticles following oral administration (Challacombe et al. 1991; Eldridge et al. 1990; O'Hagan et al. 1989). Furthermore, antigens entrapped in biodegradable microparticles have also been shown to induce systemic immunity follow-

Table III. Characteristics of an 'ideal' new vaccine

Safe, particularly in the young and in the immunocompromised
Capable of being administered orally
Easy and inexpensive to manufacture on a large scale
Induces effective immunity, preferably following a single dose
Should be capable of being administered to the very young
Heat-stable (minimal cold chain requirements)
Easily administered by untrained personnel
Should readily fit into existing national/international vaccination programmes

ing oral administration (Challacombe et al. 1991; Eldridge et al. 1990). The use of biodegradable microparticles for oral immunisation offers a number of potential advantages over alternative approaches (table II). The polymers used for preparation of microparticles are approved by the FDA for parenteral use in humans and have a well established record of safety and biocompatability. In addition, microparticles possess several of the characteristics of an 'ideal vaccine-delivery system'; these requirements are highlighted in table III. Hence, microparticles have tremendous potential as oral vaccines, but this area of research is only recently established and limited studies have been performed to date.

There are several potential problems associated with the use of microparticles as oral vaccines. For example, too little is known about the reproducibility of uptake into Peyer's patches on repeated administration, or about the potential variability among individuals. In the studies undertaken so far, uptake appears to be inefficient, although this may be improved by targeting microparticles to the GALT. Hence, particle uptake may prove to be unpredictable and difficult to exploit successfully. Finally, it should be emphasised that all the studies on particulate uptake into the GALT have been conducted in small animal models and this area of research is not without controversy. Nevertheless, some evidence does exist to demonstrate the uptake of exogenous material of the appropriate size range into the Peyer's patches of humans (Shepherd et al. 1987). Small scale studies are planned in humans in the near future to assess the ability of microparticles to function as oral antigen delivery systems for entrapped antigens.

2.3 Lectins

The ability of a range of proteins to induce immune responses following oral administration was assessed by de Aizpura and Russell-Jones (1988). They found that proteins with 'lectin or lectin-like' binding activity to glycoproteins or glycolipids in the intestinal mucosa were capable of inducing immune responses, while proteins without binding activity were not. In a subsequent study, peptide and protein antigens were conjugated to cyanocobalamin and administered orally to experimental animals (Russell-Jones & de Aizpura 1988). Cyanocobalamin is transported across the intestine by receptor-mediated endocytosis after binding to the intrinsic factor (IF) in the stomach. The IF is recognised and endocytosed by an intestinal receptor due to 'lectin-like' binding activity. The orally administered antigen-cyanocobalamin conjugates induced serum antibody responses, while the unconjugated antigens did not.

There is an extensive range of lectins from a wide variety of sources, including many from plants, that may have potential as oral adjuvants. Indeed, the adjuvant effects of CTB are thought to be mainly due to its 'lectin-like' binding properties to the GM1 ganglioside receptor on intestinal cell surfaces (GM1 gangliosides are membrane components present in all eucaryotic cells). However, a cause for concern is the reported toxicity of many lectins when administered orally to experimental animals (Pusztai 1989). Furthermore, conjugation of lectins to antigens may be required to prevent immune responses to unrelated antigens and the effects of conjugation on lectin properties need to be determined.

2.4 Liposomes

Although some success has been achieved in studies involving oral administration of antigens entrapped in liposomes (Michalek et al. 1989), disappointing results have also been reported by many workers. The stability of liposomal preparations during storage is limited and stability in the gut is a significant problem. Indeed, it has been reported that liposomes are unstable in the gut, are not taken up by epithelial cells and do not promote the absorption of entrapped drugs (Chiang & Weiner 1987). Thus, it seems unlikely that liposomal systems will find applications as oral vaccines.

2.5 Immunostimulating Complexes

An alternative novel approach to vaccine development that has aroused considerable interest in recent years is the use of immunostimulating

Table IV. Antigen delivery systems for oral immunisation

Systems	Advantages	Disadvantages
Colonisation of Peyer's patches with genetically engineered organisms (e.g. *Salmonella typhi*)	Potential for potent stimulation of immune response	Antibodies to carrier organism may preclude use for booster immunisation
Cholera toxin (B subunit)	Very potent adjuvant	Probably requires whole toxin, chemical coupling required
Microparticles	Promotes uptake by Peyer's patches, protects antigen, controlled release, can include adjuvants and targeting agents	Unproven efficacy in humans, uptake of particles requires further study
Liposomes	May include adjuvants, may partially protect antigens from degradation	Stability problems, limited efficacy shown so far
Lectins	Wide range of materials available for assessment	Nonspecific enhancement of immune responses to gut contents possible, toxicity? May require chemical coupling

complexes (Morein et al. 1984). These complexes are formed between the saponin adjuvant Quil A, cholesterol and amphipathic antigens; they have been used for parenteral immunisation in many studies since 1984. Recently, they have also been shown to be capable of inducing antibody responses following oral administration (Mowat et al. 1991). This is perhaps not surprising, since saponins alone had previously been reported to be effective oral adjuvants for associated antigens (Chavali & Campbell 1987). Nevertheless, the incorporation of antigen and saponin into a defined complex, the immunostimulating complex, may prevent induction of adverse immune responses to 'bystander' antigens. However, there are no data to indicate whether the complexes are stable in the gut or whether the saponin is released by degradation. If so, it may promote immunity to additional unrelated antigens.

3. Conclusions

In recent years, there has been a gradual acceptance of the important role of sIgA in protecting mucosal surfaces against infection with a wide range of pathogenic organisms. Since sIgA is most efficiently stimulated by local application of antigens, there has been an increasing emphasis on the role of oral immunisation. The numerous advantages of the oral route over alternative routes of administration has driven the search for effective vaccines, often despite disappointing results. Nevertheless, recent developments in recombinant DNA technology and in the formulation of antigen delivery systems have given rise to justified optimism that the next decade will see the development of several new and improved oral vaccines (table IV). These vaccines will undoubtedly come to play an important role in preventing diseases caused by a number of pathogenic organisms which are currently poorly controlled. New oral vaccines are likely to be particularly beneficial in the Third World, where intestinal infections still cause a massive toll of preventable deaths. Because of the existence of the common mucosal immune system, new oral vaccines also offer the promise of control over several sexually transmitted diseases for which there are no vaccines currently available.

References

Ambruster C, Junker W, Vetter N, Jaksch G. Disseminated bacille Calmette-Guerin infection in an AIDS patient 30 years after BCG vaccination. Journal of Infectious Diseases 162: 1216, 1990

Bergmann K-C, Waldman RH. Stimulation of secretory antibody fol-

lowing oral administration of antigen. Reviews of Infectious Diseases 10: 939-950, 1988
Besredka A. Local immunization, Williams and Wilkins, Baltimore, 1927
Bolognesi DP. Fresh pathways to follow. Nature 344: 818-819, 1990
Brochier B, Thomas I, Baudin B, Leveau T, Pastoret PP, et al. Use of vaccinia-rabies recombinant virus for the oral vaccination of foxes against rabies. Vaccine 8: 101-104, 1990
Burke KL, Dunn G, Ferguson M, Minor PD, Almond JW. Antigen chimaeras of poliovirus as potential new vaccines. Nature 332: 81-82, 1988
Challacombe SJ, Rahman D, Jeffery H, Davis SS, O'Hagan DT. Biodegradable microparticles for oral immunisation. Immunology, in press, 1991
Chatfield SN, Strugnell RA, Dougan G. Live *Salmonella* as vaccines and carriers of foreign antigenic determinants. Vaccine 7: 495-498, 1989
Chavali SR, Campbell JB. Adjuvant effects of orally administered saponins on humoral and cellular immune responses in mice. Immunobiology 174: 347-359, 1987
Chengalvala M, Lubeck MD, Davis AR, Mizutani KM-N, Morin J, et al. Evaluation of adenovirus type 4 and type 7 recombinant hepatitis B vaccines in dogs. Vaccine 9: 485-491, 1991
Chiang C-M, Weiner N. Gastrointestinal uptake of liposomes I: in vitro and in situ studies. International Journal of Pharmaceutics 37: 75-85, 1987
Clements JD. Use of attenuated mutants of salmonella as carriers for delivery of heterologous antigens to the secretory immune system. Pathological and Immunological Research 6: 137-146, 1987
Curtiss III R, Kelly SM, Gulig PA, Nakayama K. Selective delivery of antigens by recombinant bacteria. Current Topics in Microbiology and Immunology 146: 35-50, 1989
de Aizpura HJD, Russell-Jones GJ. Oral vaccination: identification of classes of proteins that provoke an immune response upon oral feeding. Journal of Experimental Medicine 167: 440-451, 1988
Eldridge JH, Hammond CJ, Meulbroek JA, Staas JK, Gilley RM, et al. Controlled vaccine release in the gut associated lymphoid tissues I: orally administered biodegradable microspheres target the Peyer's patches. Journal of Controlled Release 11: 205-214, 1990
Evans DMA, Dunn G, Minor PD, Schild GC, Cann AJ, et al. Increased neurovirulence is associated with a single nucleotide change in a non-coding region of the Sabin type 3 polio vaccine genome. Nature 314: 548-550, 1985
Fairweather NF, Chatfield SN, Makoff AJ, Strugnell RA, Bester J, et al. Oral vaccination of mice against tetanus by use of a live attenuated Salmonella carrier. Infection and Immunity 58: 1323-1326, 1990
Forrest BD, LaBrooy JT, Robinson P, Dearlove CE, Shearman DSC. Specific immune response in the human respiratory tract following oral immunization with live typhoid vaccine. Infection and Immunity 59: 1206-1209, 1991
Hackett J. Salmonella-based vaccines. Vaccine 8: 5-11, 1990
Hale TL, Formal SB. Oral shigella vaccines. Current Topics in Microbiology and Immunology 146: 205-211, 1989
Holmgren J, Clemens J, Sack DA, Svennerholm A-M. New cholera vaccines. Vaccine 7: 94-96, 1989
Holmgren J, Svennerholm A-M. New vaccines against bacterial enteric infections. Scandinavian Journal of Infectious Diseases 70 (Suppl.): 149-156, 1990
Levine MM, Herrington D, Murphy JR, Morris JG, Losonsky G, et al. Safety, infectivity, immunogenicity, and *in vivo* stability of two attenuated auxotrophic mutant strains of salmonella typhi, 541Ty and 543Ty, as live oral vaccines in humans. Journal of Clinical Investigation 79: 888-902, 1987
Lindberg AA, Kärnell A, Stocker BAD, Katakura S, Sweiha H, et al. Development of an auxotrophic oral live *Shigella flexneri* vaccine. Vaccine 6: 146-150, 1988
Lycke N, Holmgren J. Strong adjuvant properties of cholera toxin on gut mucosal immune responses to orally presented antigens. Immunology 59: 301-308, 1986
Lycke N, Holmgren J. Long term cholera antitoxin memory in the gut can be triggered to antibody formation associated with protection within hours of an oral challenge immunization. Scandinavian Journal of Immunology 25: 407-412, 1987
McGhee J, Mestecky J. In defense of mucosal surfaces. Infectious Disease Clinics of North America 4: 315-341, 1991
Mestecky J, McGhee JR. Immunoglobulin A (IgA): molecular and cellular interactions involved in IgA biosynthesis and immune response. Advances in Immunology 40: 153-245, 1987
Michalek SM, Childers NK, Katz J, Denys FR, Berry AK, et al. Liposomes as oral adjuvants. Current Topics in Microbiology and Immunology 146: 51-58, 1989
Minor PD, John A, Ferguson M, Icenogle JP. Antigenic and molecular evolution of the vaccine strain of type 3 poliovaccine during the period of excretion by a primary vaccinee. Journal of General Virology 67: 693-706, 1986
Morein B, Sundquist B, Hoglund S, Dalsgaard K, Osterhaus A. Iscom, a novel structure for antigenic presentation of membrane proteins from enveloped viruses. Nature 308: 457-460, 1984
Mowat AMcI, Donachie AM, Reid G, Jarrett O. Immune stimulating complexes containing Quil A and protein antigen prime class I MHC-restricted T lymphocytes in vivo and are immunogenic by the oral route. Immunology 72: 317-322, 1991
O'Hagan DT, Palin KJ, Davis SS, Artursson P, Sjoholm I. Microparticles as potentially orally active immunological adjuvants. Vaccine 7: 421-424, 1989
O'Hagan DT. Novel nonreplicating antigen delivery systems. Current Opinion in Infectious Diseases 3: 393-401, 1990a
O'Hagan DT. Intestinal translocation of particulates: implications for drug and antigen delivery. Advanced Drug Delivery Reviews 5: 265-295, 1990b
Owen RL, Ermak TH. Structural specializations for antigen uptake and processing in the digestive tract. Springer Seminars in Immunopathology 12: 139-152, 1990
Pusztai A. Lectins. In Cheeke (Ed.) Toxicants of plant origin: Vol. III, Proteins and amino acids, pp. 29-71, CRC Press, Boca Raton, 1989
Renegar KB, Small Jr PA. Passive transfer of local immunity to influenza virus infection by IgA antibody. Journal of Immunology 146: 1972-1978, 1991
Russell-Jones GJ, de Aizpura HJD. Vitamin B12: a novel carrier for orally presented antigens. Proceedings of the International Symposium on Controlled Release of Bioactive Materials 15: 142-143, 1988
Shepherd NA, Crocker PR, Smith AP, Levison DA. Exogenous pigment in Peyer's patches. Human Pathology 18: 50-54, 1987
Stover CK, de la Cruz VF, Fuerst TR, Burlein JE, Benson LA, et al. New use of BCG for recombinant vaccines. Nature 351: 456-460, 1991
Sussman M, Collins CH, Skinner FA, Stewart-Tull DE (Eds). The release of genetically-engineered micro-organisms, Academic Press, 1989
Tacket CO, Forrest B, Morona R, Attridge SR, LaBrooy J, et al. Safety, immunogenicity and efficacy against cholera challenge in humans of a Typhoid-Cholera hybrid vaccine derived from *Salmonella typhi* Ty21a. Infection and Immunity 58: 1620-1627, 1990
Wilson AD, Clarke CJ, Stokes CR. Whole cholera toxin and B subunit act synergistically as an adjuvant for the mucosal immune response to keyhole limpet haemocyanin. Scandinavian Journal of Immunology 31: 443-451, 1990
Witebsky E. Immunology: views of the past and visions of the future. Canadian Medical Association Journal 97: 1371-1377, 1967
World Health Organisation. Potential use of live viral and bacterial vectors for vaccines. Vaccine 8: 425-437, 1990

Correspondence and reprints: Dr *D.T. O'Hagan*, Department of Pharmaceutical Sciences, University of Nottingham, University Park, Nottingham NG7 2RD, England.