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Innovations in oral gene delivery: challenges and potentials

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The oral delivery of peptide, protein, vaccine and nucleic acid-based biotechnology products is the greatest challenge facing the drug delivery industry. Oral delivery is attractive due to factors such as ease of administration, leading to improved patient convenience and compliance, thereby reducing overall healthcare costs. The realization that gene therapy will provide a tangible and potentially huge new therapeutic opportunity has stimulated interest in oral gene delivery. Here we summarize the oral gene delivery vehicles currently in use and highlight potential areas of application, along with the challenges that need to be overcome before this new technology enters the clinic.

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▼ The possibility of curing a genetic disease by swallowing a magic pill containing a correct copy of the defective gene is a scenario that appeals to us all, but how far are we from realising this ultimate aim? Although it is certainly a more long-term solution, oral gene delivery is nonetheless one goal towards which numerous biotechnology companies are currently striving. The main advantages presented by oral gene delivery are the ease of target accessibility, enhanced patient compliance owing to the non-invasive delivery method, and the possibility of local and systemic gene therapy. In the case of DNA vaccines, oral delivery might also stimulate mucosal immunity when presented in the correct format.

The past 25 years have seen almost universal acceptance of the concept of gene therapy. The field started with much fanfare and overly optimistic expectations in the 1980s and early 1990s. By the middle of the 1990s, it was becoming apparent that therapeutically useful gene therapy applications in humans would be more difficult than envisaged because the data emerging from clinical trials were much less convincing than expected. The field of gene therapy received another severe setback with the death of a patient on a clinical trial in 1998 owing to an adverse immune reaction to the high dose of adenoviral vector that was used to administer the gene encoding ornithine transcarbamaylase[1].

Despite these setbacks, a success earlier this year in gene therapy has very clearly demonstrated its tremendous potential. The group of Alain Fischer successfully treated two infants with severe combined immunodeficiency[2] (SCID-XI) by transfecting bone marrow stem cells with a normal copy of the cytokine receptor γ chain. These patients were able to leave protective isolation, discontinue treatment and appear to be growing and developing normally.

To date, most gene delivery strategies have concentrated on the parenteral route of delivery and oral administration has been largely ignored. This is mainly due to the large hurdles that need to be overcome for oral gene delivery, such as the acid pH in the stomach (which leads to depurination of the DNA), the nucleases, lipases and peptidases present in the GI tract, and the poor permeability of both genes and gene vectors across the intestinal epithelium owing to the size and charge of the gene delivery vehicles. As a result of these factors, the greatest challenge faced by oral gene therapy is achieving delivery of sufficient genetic material in the correct cell types to produce therapeutic or prophylactic protein expression levels.

The two main areas of application for oral gene delivery are corrective gene therapy (both local and systemic) and genetic mucosal immunization via the Peyer's Patches, the immune sampling portals that occur in discrete patches in the small intestine. As it is estimated that oral drug products account for half of the annual drug delivery market, it is

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realistic to assume that oral gene delivery will become increasingly fruitful from both therapeutic and economic perspectives. Oral drug delivery has been reviewed recently[3]. This article discusses oral gene delivery vectors and current innovative technologies that should improve delivery of genes across the GI tract.

Potential applications of oral gene delivery

Gene therapy

Despite the fact that somatic gene therapy to the intestine was suggested in 1992 (Ref. 4), it has not been seriously investigated until recently. This was primarily because of the low oral bioavailability of the available DNA vector systems and also the relatively few genetic disorders directly associated with the GI tract (e.g. familial adenomatous polyposis, cystic fibrosis and various colon cancers). Other intestinal diseases that could directly benefit from oral gene delivery include inflammatory bowel disease and Crohn's disease.

The intestinal epithelium consists of a large pool of continuously cycling cells derived from the crypts of Lieberkühn, which present a large cellular surface area for transduction with a therapeutic gene[5]. The gut epithelium is a highly vascularized tissue with the capillaries very close to the epithelial cell layer, which allows the secretion of encoded proteins from the epithelium into the systemic circulation[4] (Fig. 1a). This proximity might also allow the access of exogenous DNA or DNA delivery vectors to many organs via the systemic system following transcellular (via transcytosis) or paracellular (via temporary disruption of tight junctions) delivery across the GI epithelium. Because the intestine shares many enzymatic pathways with the liver, DNA delivery to the GI tract has been proposed for the genetic correction of metabolic

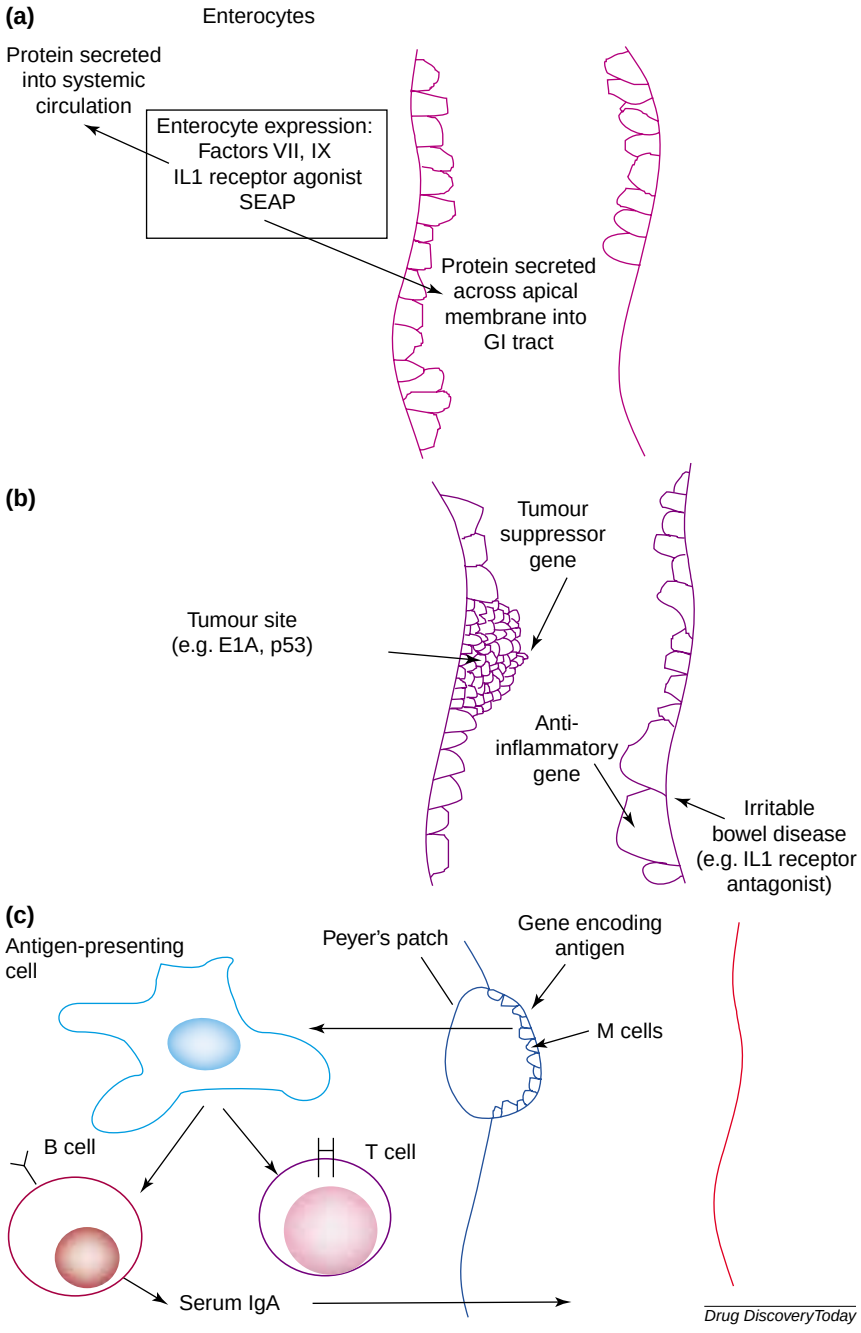

Figure 1. Potential applications of oral gene delivery. **(a)** Systemic gene delivery – the enterocytes are used as factories to produce soluble protein that can be secreted into the systemic circulation (e.g. the clotting Factors VIII and IX) or across the apical membrane into the gastrointestinal (GI) tract, where the protein can act locally or distally (e.g. secreted alkaline phosphate). **(b)** Local gene therapy for diseases of the intestine (e.g. inflammatory bowel disease, GI tumours) – therapeutic genes (e.g. tumour suppressor and anti-inflammatory genes) could be delivered to the affected site. The disease states of these tissues might also allow targeting because of different receptor expression on the apical membrane. **(c)** DNA vaccines are a particularly attractive goal for intestinal gene delivery owing to the potential induction of mucosal immunity. In this case, the gene encoding the antigen of interest is taken up by the M cells of the Peyer's patch if presented as part of a particulate system. Uptake of the gene delivery vector might be enhanced by the addition of targeting ligands that facilitate uptake by the M cells.

Table 1. DNA delivery methods that have been used for oral gene therapy

Delivery vehicle	Examples	Major areas for development
Polymers	PLGA/PLA copolymers Chitosan Fumaric acid/sebacic acid copolymers	Targeting by surface modification of particles; use of various chemical enhancers, mucolytic agents for improved bioavailability; vary DNA release rates depending on specific application
Cationic lipids	DOTAP	Increase stability in gastrointestinal environment; targetting by surface modification of particles
Recombinant viruses	Adenoviruses, retroviruses, adeno-associated virus, vaccinia virus, plant-derived viruses	Increasing concentration of viral titres and making production more cost effective; tissue specific targetting; increased tissue-specific expression and secretion of viral-encoded transgene to the circulation
Recombinant live bacteria[a]	DNA Vaccines (*Shigella* spp, *Salmonella* spp.)	Abolish potential for reversion to virulence by generating highly attenuated vector strains; generate protective immunity in humans based on animal models
Particle bombardment to buccal mucosa	Gene gun, intraoral jet injection	Optimize delivery strategies to minimize tissue damage

[a]Live bacteria as delivery vehicles for recombinant subunit vaccines were not surveyed in this review because the antigens are expressed via bacterial transcription and translation mechanisms.
Abbreviations: DOTAP, [N-(2,3-dioleoyloxy)propyl]-N,N,N-trimethyl ammonium salts; PLGA, poly(lactide-*co*-glycolide); PLA, poly(L-lactide).

disorders, such as ornithine transcarbamylase deficiency[6]. Another potential application is the use of transfected epithelial cells as factories for the expression of heterotropic proteins, such as the clotting factors Factor VIII and Factor IX, which are secreted into the circulation[7].

Gene vaccines

From the initial concept of vaccination with naked DNA[8], gene vaccines have evolved into a realistic possibility, with several clinical trials[9]. In addition to the relative ease and low cost of manufacture, another advantage of nucleic acid vaccines is that they mimic a natural infection because the encoded antigen is presented in the context of major histocompatibility complex class I and II molecules. As well as systemic immunity, assuming access to the microfold (M) cells covering the Peyer's patch, which can attach microorganisms, oral gene vaccines are capable of eliciting a mucosal response (Fig. 1c). This provides an important first line of defence against pathogens that infect via the mucosae of the GI, genitourinary or respiratory tracts[10,11].

The oral delivery route is very attractive because the GI tract has over 300 m^2 of mucosal surface that is richly endowed with immune inductive tissue, such as the intraepithelial lymphocytes and lymphoid follicles of the lamina propria and Peyer's patches, collectively known as the gut-associated lymphoid tissues (GALT). Many different delivery vehicles have been investigated for the oral delivery of gene vaccines, including viral and synthetic vectors, and bacterial delivery systems, all of which are described further below.

Oral gene delivery systems

Gene delivery technology can be broadly divided into the following categories: (1) viral vectors; (2) synthetic delivery systems, which include polymer-based systems and lipids; (3) physical methods; and (4) for gene vaccines, bacterial delivery systems (Table 1; Fig. 2). In Table 1 we list some of the ways in which these various delivery systems could be improved in order to facilitate proceeding to clinical trials. To date, the viral vectors have proved to be the most efficient gene delivery vehicles and also show the potential for long-term gene expression because several of the viral systems promote integration of the delivered gene into the host chromosome.

The limitations of viral vectors include the immunogenicity of the viral vector itself and, in some cases, limited DNA packaging capacity. By contrast, synthetic delivery systems are less immunogenic[12], thereby providing the potential to re-administer the gene, theoretically have unlimited packaging capacity, are relatively cheap and easy to produce, and demonstrate long-term stability and ease of storage. In addition, synthetic vectors are more flexible and easier to tailor to the application (e.g. the level of mucoadhesion of the polymer, conjugation of targeting

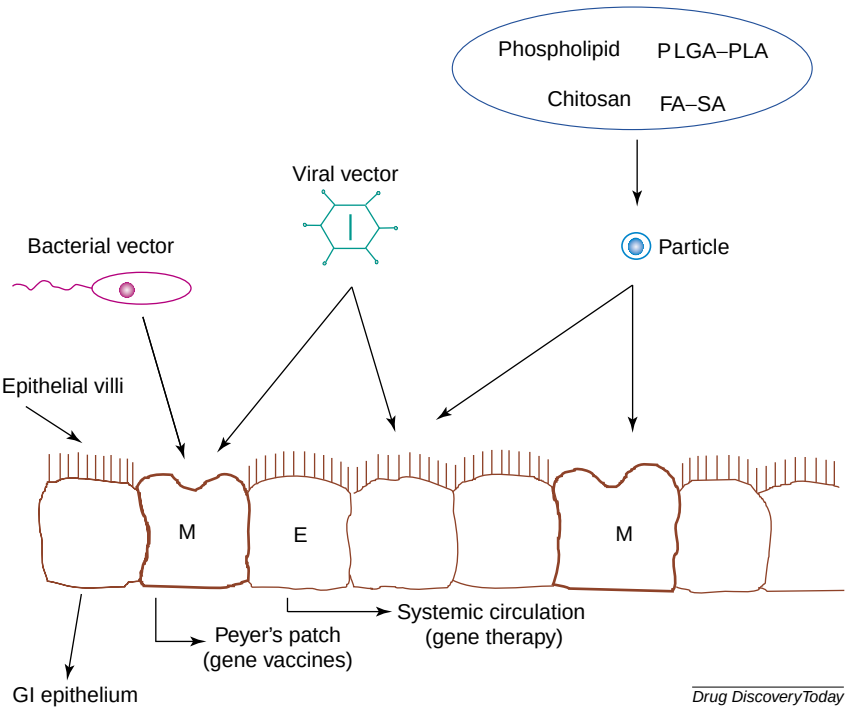

Figure 2. Vector-mediated oral gene delivery systems. Bacterial vectors have been used to deliver their extrachromosomal DNA cargo by specifically infecting M cells (M), leading to expression of the antigenic protein. Viral vectors are used as both gene therapy vehicles to transfect epithelial cells (E) directly and as gene vaccines via M cells. Various synthetic polymers have been used to condense DNA into particles for delivery to gut epithelial and M cells. Abbreviations: FA–SA, copolymers of fumaric acid and sebacic acid; GI, gastrointestinal; PLGA–PLA, copolymers of poly(lactide-*co*-glycolide) and poly(L-lactide).

moieties). However, the transduction efficiency of these systems, although it is improving all the time, does not yet match that of viral systems.

Polymer-based gene delivery

Poly(lactide-*co*-glycolide) (PLGA) and poly(L-lactide) (PLA) polyesters, which have FDA approval for drug implants, are widely used for the production of biodegradable and biocompatible micro- and nanoparticles. Although they cannot themselves transduce cells, the particles are phagocytosed by M cells in the Peyer's patches, making them a useful polymer system for oral vaccination. Several early studies suggested that particles of up to 10 μm diameter could be phagocytosed by M cells[13] but the efficiency appears to be considerably better with smaller particles[14] (~100 nm). The production of significant serum IgG, IgM and IgA, and secretory IgA (sIgA) in mucosae has been demonstrated using PLGA delivery systems[15].

Two additional studies showed a protective immune response against rotavirus infection in Balb/c mice (an inbred white mouse strain that is much used by immunologists because of its defined immunological response) following oral gavage with PLGA-encapsulated plasmid DNA encoding rotaviral proteins[16,17]. It was shown that, in comparison with the gene-gun administration of DNA, which necessitated two doses, only one inoculation was needed when PLGA-encapsulated DNA was given orally, although considerably greater quantities of DNA were required for oral delivery. Mucosal and systemic immune responses have been generated in Balb/c mice following oral administration of PLGA-encapsulated DNA encoding the HIV envelope glycoprotein, gp160 (Ref. 18). Continuing development of the PLGA encapsulation technology has shown that 60% encapsulation efficiency of DNA can be achieved with release rates that vary depending on the formulation. In addition, the DNA released from the PLGA complexes appears to retain considerable stability, as judged by the degree of supercoiling[19–21]. However, it might be subject to some acid hydrolysis in the microenvironment of the PLGA particle as it degrades itself.

Chitosan is a natural biodegradable mucoadhesive polysaccharide derived from crustacean shells. This slowly degradable polymer has been shown to increase transcellular and paracellular transport of macromolecules across intestinal epithelial monolayers[22]. Already available in pill form as an alternative therapy to reduce dietary fat and cholesterol absorption[23], chitosan has more recently been used successfully to deliver a reporter gene (encoding chloramphenicol acetyl transferase) orally to enterocytes, Peyer's patches and mesenteric lymph nodes[24]. Another study indicated that mice fed chitosan–DNA particles expressed the reporter gene *lacZ* (encoding bacterial β-galactosidase) 5 days after administration[10]. This study also showed that orally administered chitosan–DNA complexes can stimulate an immune response to the principal peanut allergen Arah-2. Mice fed Arah-2–chitosan nanoparticles had increased levels of sIgA in faecal extracts, indicating mucosal immunity. There was also an increase in serum IgG2a, indicating a type-1 T-helper (T_{H1}) cell-mediated immune response. A protective immune response was generated against peanut allergy following the oral administration of Arah-2–chitosan complexes.

Copolymers of the biodegradable polyanhydrides fumaric acid (FA) and sebacic acid (SA) have also been used

to deliver DNA orally[25]. Studies with an easily traceable marker, colloidal gold, suggested that the poly(FA–SA) particles were translocated across the mucosal epithelium and the follicle-associated epithelium covering the Peyer's patches by both transcellular and paracellular routes as soon as one hour after feeding[25]. Microspheres were also observed in the spleen and liver. The visualization of particles within cells indicated their transduction competency but the mode of entry was not clear. In rats fed poly(FA–SA) loaded with the *lacZ* reporter gene, levels of β-galactosidase activity in the small intestine, Peyer's patches and liver increased twofold compared with rats fed unencapsulated DNA. Thus, this poly(FA–SA) copolymer could be used to transduce cells of the GI tract, as well as cells in the Peyer's patches, and gene delivery to the liver might also be feasible following uptake across the GI tract.

Lipid-based gene delivery vehicles

Although they are widely used for parenteral gene delivery, cationic lipid-based systems are not very popular for oral gene delivery. This is mainly due to their instability in the gut environment. Etchart and colleagues fed 100 μg of naked DNA encoding the measles virus haemagluttinin (HA) by oral gavage to mice, and this induced a weak splenic cytotoxic T-cell (CTL) response[26]. However, when the DNA was coadministered with an adjuvant, cholera toxin or dioleyl-1,2-diacyl-3-trimethylammonium-propane liposomes, the anti-HA CTL response increased significantly. Similar results were obtained after a transepithelial injection into the jejunum.

Viral delivery systems

Viral vectors take advantage of the natural infectivity inherent in the structure of the wild-type virus but are altered to minimize or abolish their replicative and lytic functions. A variety of viral vector systems have been used to deliver DNA to intestinal tissue for corrective gene therapy and also to generate mucosal immunity (Table 2).

Retroviruses Retroviruses were the first viruses to be investigated as vectors for intestinal gene therapy because they only transfect M-phase cells. This is particularly relevant because of the proliferative nature of GI epithelial tissue. Initial experiments with retroviruses proved the concept

Table 2. Advantages and disadvantages of each of the most commonly used live vectors

Live oral vector type	Application	Advantages	Disadvantages	Refs
Retrovirus	Gene therapy	Tropism for infecting rapidly dividing cells (i.e. enterocytes); long-term transgene expression	Relatively low viral titres; poor efficiency of transfection	5,23–25
Adenovirus	Gene therapy, recombinant subunit vaccine	High viral titres; enterotropic serotype identified, efficient transduction of enterocytes	Short duration of peak transgene expression levels (up to 7 days); inflammatory and immunological response may preclude repeat administration; concerns regarding toxicity	27–40
Adeno-associated virus	Gene therapy, recombinant subunit vaccine	High viral titres, long-term transgene expression; efficient transduction of enterocytes; reduced immunogenicity allowing repeat administration	Relatively small DNA packaging capacity	41–49
Vaccinia virus	Recombinant subunit vaccine	Protective mucosal immunity in animal models	Short duration of expression; inefficient delivery for corrective gene therapy	50,51
Plant-specific viruses	Recombinant subunit vaccine	High levels and fast onset of transgene expression; cheaper and easier to generate high viral titres	Inefficient delivery for corrective gene therapy; inflammatory and immunological responses may preclude repeat administration	52
Enterobacteria	DNA vaccination	Natural tropism of vector for GALT; efficient delivery in animal models	Safety risks due to potential reversion to virulence	59–62

of using viral vectors for DNA delivery to GI epithelia in animal models. High levels of reporter gene expression *in vitro* in colon cell models were reported, with significant levels of retrovirus-encoded transgenes (Factor VIII and Factor IX) secreted through the basolateral layer[7].

Low *in vivo* transfection efficiencies have so far limited the use of retroviruses for oral gene therapy[27,28]. However, cleaner production processes and the ability to generate more potent retroviral vector preparations have recently been reported[29]. In addition, vector systems derived from lentiviruses (a subgroup of the retrovirus family) have been shown to transfect both dividing and nondividing cells successfully[30]. It is not yet known whether or not these recent advances in vector technology will address the limitations of retroviral delivery to the intestine and promote retroviral vectors as rational contenders for successful gene therapy of the gut.

Adenoviruses Recombinant adenoviruses are the most common form of viral vectors currently in use for gene therapy applications. Many different adenoviral serotypes have been identified, with types 2 and 5 (Ad2 and Ad5) being the most highly characterized and the most commonly used in gene therapy protocols to date. The dilution effect within the GI tract necessitates the use of higher viral titres, making adenoviruses attractive as vectors for oral gene delivery owing to the ability to produce high-titre preparations[31].

Recently, an enterotropic adenovirus serotype, Ad41, has been identified as having a much higher tropism for differentiated intestinal epithelial cells than Ad5 and has been proposed as the first tissue-specific vector for intestinal gene delivery[32]. Adenoviral vectors have been shown to transfect gut epithelial cells more efficiently than retroviruses but the duration of adenovirus-mediated transgene expression is generally considerably shorter because they do not insert their genome into the chromosome of the host cell[33,34].

Adenovirus-transduced gut epithelial cells have been shown to secrete a transgene-encoded protein, secreted alkaline phosphatase (SEAP) into the bloodstream *in vivo* and the interleukin-1 (IL-1) receptor antagonist through the basolateral layer *in vitro*, establishing the potential of enterocytes to act as 'factories' for the synthesis of heterologous gene products[35,36]. Wirtz and colleagues[37] have shown that adenovirus-mediated transduction of colonic epithelial cells *in vivo* is more efficient in mice with experimental colitis than in untreated mice, and have also reported reduced inflammatory or adverse immunogenic side effects, which are normally associated with adenoviruses. These findings suggest the value of using encapsulated adenoviruses for controlled release within the colon.

In addition to their use as vehicles for corrective gene therapy, adenoviruses have been widely used to deliver heterologous genes encoding antigenic proteins via the oral route in order to generate mucosal immunity[38–44]. Despite showing greatest promise as an oral vector for gene delivery to the intestine, adenoviral vectors will require further improvements in the efficiency of transgene delivery in order to achieve therapeutic benefit. Improvements will require increasing the concentration of the expressed transgene, prolonging the duration of expression, enhancing tissue-specific targeting (e.g. with Ad41), optimizing expression from episomal viral genomes and reducing inflammatory and immunological side-effects associated with repeated adenovirus administration. However, in the case of gene vaccines, repeated administration might not be a significant issue.

Adeno-associated viruses Current adeno-associated virus (AAV) vectors cannot replicate but retain the ability to infect both dividing and nondividing cells[45,46]. AAV vectors are less immunogenic than adenoviruses or retroviruses, allowing repeat administration where single dose administration is not feasible. AAV vectors have been intensively studied for gene delivery but have only recently emerged as viable alternatives for routine gene therapy in clinical trials, as a result of improvements in viral vector production[47,48]. A significant advantage provided by AAV vectors is the long duration of transgene expression, possibly due to the integration of the AAV genome containing the heterologous gene into the host chromosome.

An AAV vector carrying the *lacZ* gene has been reported to correct the phenotype of a rat model of lactose intolerance after oral administration. Progenitor stem cells, enterocytes and lamina propria cells expressed the transgene for up to six months[49]. More recently, similar levels of delivery and expression in the intestine were reported when an AAV vector encoding the gene for the NR1 subunit of the *N*-methyl-D-aspartate receptor was administered as an oral vaccine to rats, generating neuroprotection against experimental stroke and epilepsy[50].

No direct comparisons of AAV and adenovirus DNA delivery to intestinal tissue have yet been reported. However, such studies in other tissues suggest that AAV vectors are more effective DNA delivery vehicles owing to their reduced immunogenicity and longer duration of transgene expression[51]. Traditionally, AAVs have been restricted in their use as vectors for gene therapy of disorders requiring the delivery of large genes (>5 kb) owing to their limited packaging capacity. However, recent studies have shown that this limitation can be overcome[52,53], which means that AAV vectors have strong potential as effective agents

for corrective oral gene therapy and genetic vaccines. As such, they should take a leading role as the viral vector of choice for DNA delivery to the intestine.

Other viral systems Many recombinant viral systems have been developed as vectors for generating mucosal immune responses[54] but only a few of these have been used to deliver heterologous antigens via the oral route[11,55]. This might reflect difficulties associated with ensuring safe and efficient delivery to the GALT within the intestine and the need to generate the relatively high titres of novel recombinant vector that are required for oral delivery. The most widely studied of these are the poxviruses, including the prototype vaccinia virus (VV). Gherardi and Esteban have recently demonstrated mucosal and systemic immune responses to both vector-specific antigens and the encoded recombinant products from orally administered VV vectors in a murine model[11]. This study also detected expression of VV-encoded reporter genes in the GALT and spleen, indicating access to the systemic system via the lymphoid tissue of the intestine.

Plant-specific viruses have also been evaluated as recombinant oral gene delivery vectors to induce immune responses to the expressed antigen product. These viral vectors generate optimal responses when encased within plant cells, where they are afforded additional protection from the gastric environment[55]. Other examples of these potential recombinant oral systems include poliovirus, which has a natural tropism for gut epithelial cells[56], Semliki Forest virus[57], Sindbis virus[58] and Venezuela equine encephalitis virus[59]. New advances in specific viral vector production techniques (as for AAV) and further refinements in delivery strategies to epithelial cells (e.g. polycation-enhanced delivery[60]) should allow conventional recombinant virus systems to emerge as viable oral gene delivery vectors.

Bacterial delivery systems

Recombinant bacteria are efficient vehicles for the delivery and display of heterologous antigens for oral vaccination[61]. Recently, recombinant strains of *Salmonella* and *Shigella* have been used to deliver DNA vaccines to the GALT[62–64]. After infection, *Shigella* vectors undergo programmed autolysis within the host cell, delivering the DNA vaccine cargo into the cytoplasm[65]. *Salmonella* vectors are understood to act by inducing apoptosis of the infected host cell, resulting in delivery of the DNA vaccine to the nearby dendritic cells[64].

Particle bombardment

Unlike the above methods for oral gene delivery, direct physical bombardment does not involve directly accessing cells of the GI tract. This is a novel method for the rapid, direct immunization of easily accessible mucosal sites, such as the buccal mucosa. Ballistic gene delivery is achieved by coating the plasmid DNA onto small gold beads (micrometre diameters) that are accelerated into the target tissue using a helium blast. The small size of the gold beads ensures minimal tissue damage.

Keller and colleagues used this method to deliver genes encoding the cytokines IL-2, IL-6 and granulocyte macrophage-colony stimulating factor (GM-CSF) to the buccal mucosa of dogs[66]. The cytokines could be detected in the oral mucosa for up to 4 days after administration but not in serum. Another study compared three different methods of gene delivery in the mouth using HIV DNA – gene-gun delivery, intraoral jet injection (using a high-pressure jet injector used in paediatric dentistry) and intramuscular injections of the tongue[67]. Mucosal immunization of mice by jet injection was the most efficient at inducing mucosal IgA antibodies and systemic IgG2a antibodies. Intramuscular and gene-gun administration led to a predominantly humoral type-2 T-helper cell response.

Systems to improve oral gene delivery

Targeted gene delivery

The addition of targeting ligands to the surface of a gene delivery vehicle is one means of enhancing gene delivery through receptor-mediated endocytosis. Receptors that trigger endocytosis upon ligand binding have been identified in the GI tract, which might allow the various regions of the GI tract to be targeted (e.g. enterocytes for local gene expression or M cells in the Peyer's patches for genetic vaccination). A major issue, however, is the species differences in apical receptor expression in the GI tract, which is hard to resolve in view of limited access to human GI tissue and Peyer's patches.

Many enteric pathogens infect the host following attachment to receptors on the M cells of intestinal Peyer's patches. Reovirus is one such pathogen that exploits M cells as a means to gain entry to the host, mediated by reovirus adhesin, protein σ1. This adhesin was covalently bound to polylysine and used to form a protein-σ1–polylysine–DNA complex. *In vitro*, this could transfect cells that expressed the reovirus receptor; *ex vivo*, it could transfect nasal-associated lymphoid tissue, which might have an analogous M-cell structure to GALT[68]. Three species of the bacterial genus *Yersinia* also target M cells, partly through interaction of their invasin protein with β1 integrins. This integrin subclass is expressed on the apical and basolateral membranes of M cells but only on the basolateral surface of enterocytes. Thus, β1 integrins might be selective targets for M cells and *Yersinia* invasin-derived targeting ligands might be developed that increase delivery to M cells[69].

The B subunit of cholera toxin (CTB) has a high affinity for the G_{M1} ganglioside, a glycolipid receptor present in the membrane of all nucleated cells. When CTB was bound to the surface of antigen loaded liposomes, it was shown to increase the mucosal IgA and serum IgG levels after oral administration to mice[70]. Thus, although G_{M1} is not specific for M cells, it is thought that this ganglioside is more accessible on M cells than on neighbouring enterocytes owing to the thinner glycocalyx covering M cells. This approach could prove to be very useful for the targeting of DNA vaccines to M cells.

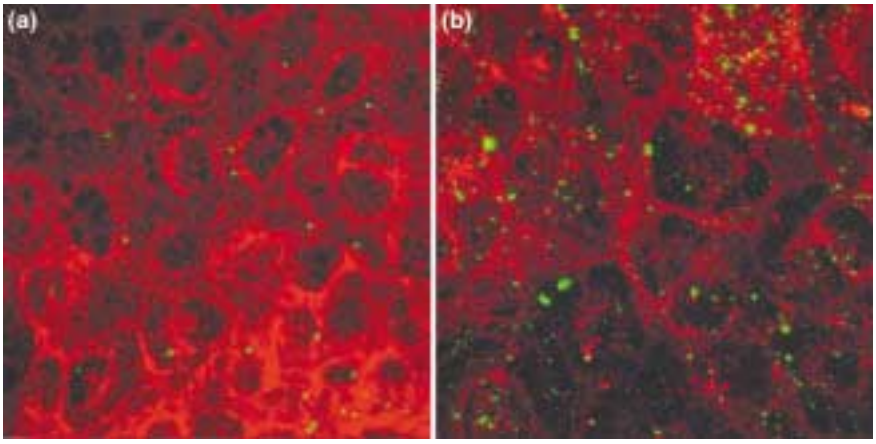

Figure 3. Increased uptake of polystyrene particles upon addition of a membrane translocation sequence (MTS). **(a)** A confocal micrograph of a negative control Caco-2 monolayer that has been exposed to polystyrene particles conjugated to bovine serum albumin. There is very low uptake of these polystyrene particles. **(b)** When these particles are conjugated with an MTS ligand, the uptake is considerably increased (J. Dee *et al.*, unpublished).

Lectin modification of liposomes and polymer microspheres for targeted drug delivery via glycoproteins of the GI tract has been extensively studied[71]. The *Ulex europeas* 1 lectin, which is derived from gorse, targeted Peyer's patches effectively in mice when incorporated into liposomes, with binding to M cells seen within 10 min of inoculation into mouse gut loops[72,73]. The lectin from elder bark (*Sambucus nigra*) has been shown in preliminary studies to bind to the apical surface of follicle-associated epithelial cells in fixed sections from human ileum[74]. Although there is considerable species variation, which makes investigations more difficult, lectins might be useful for targeting M cells and enterocytes.

In addition to targeting specific receptors, cell uptake can also be increased by non-specific peptides called membrane transduction sequences (MTS), which can cross biological membranes efficiently[75]. These include the herpes virus 22 protein VP22, the tat protein of HIV, the pre-S2 domain of hepatitis B surface antigen and hydrophobic regions of signal sequences. When an MTS peptide derived from the hydrophobic region of a signal sequence is conjugated to the surface of particles, it can increase the uptake of polystyrene particles into the intestinal model cell line Caco-2 (J. Dee *et al.*, unpublished; Fig. 3).

Enhancers

Chemical enhancers are used to promote drug absorption across the GI tract in a transient manner but the mode of action of these enhancers is not well understood. Very few of these chemicals have been tested for enhancement of gene delivery across the GI epithelium. Positively charged cyclodextrins have already been used to increase the rectal absorption of morphine[76]. They were used in combination with an adenovirus, which is a poorer mediator of transfection in differentiated enterocytes than in other cell types because they have fewer α_v integrin receptors on the apical surface of the epithelium. The cyclodextrins enhanced the attachment and internalization of adenovirus in differentiated Caco-2 monolayers and the rat jejunum, possibly by acting as viral dispersants. In doing so, it prevented virion aggregation and increased the number of viral particles that came in contact with enterocytes[77].

Other enhancers that have been used to improve oral or rectal drug delivery include protease inhibitors such as bacitracin[78], bile salts[79] and various surfactants[80]. Another enhancer, which has been approved in Japan for rectal drug administration, is the fatty acid sodium caprate, which is thought to act as a combined surfactant and membrane fluidizer[81].

Mucolytic agents

The mucous layer lining the intestine constitutes a significant barrier to oral gene delivery both to the intestinal stem cells in the crypts of Lieberkühn and the Peyer's patches. Mucolytic agents have been investigated as a means of increasing the efficiency of oral gene delivery. The reducing agents dithiotreitol and *N*-acetyl cysteine (NAC) have both demonstrated up to 75% mucous reduction, especially in combination with distention of the rat intestine[82]. A subsequent study showed that a retrovirus vector was resistant to incubation in low levels of NAC, indicating that a NAC wash before viral incubation might be feasible[83]. Thus, pretreatment with mucolytic agents might increase gene delivery to the intestine but further studies in the area will be required.

Conclusion

Using the intestine as a portal for introducing corrective or therapeutic genes into the body holds great potential benefit and is actively being investigated by many groups in both the academic and the biotechnology sectors. As with most other gene therapy applications, the major limiting factor is the poor efficiency of delivering the DNA so that the transgene is correctly expressed in the specific target tissue, in the right quantity and at the correct time. Oral gene therapy presents some uniquely challenging delivery hurdles that are being investigated both in the context of increasing the bioavailability of macromolecules in general (e.g. particulate delivery systems) and for improving established gene therapy delivery technologies (e.g. enterotropic adenoviruses). DNA vaccination is currently the most promising gene system for moving oral gene delivery technologies into the clinic. Furthermore, advances in enhancing DNA delivery to intestinal epithelial cells have raised expectations for systemic and local gene therapy applications.

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