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Manipulating the immune system: humoral versus cell-mediated immunity

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Abstract

Many of the vaccines in use today were designed on an empirical basis with little understanding of the mechanism of protective immunity or knowledge of the protective antigens. Certain of these vaccines, based on killed or attenuated bacteria or viruses, are associated with unacceptable side-effects. New generation vaccines based on recombinant proteins or naked DNA have considerably improved safety profiles, but are often poorly immunogenic, especially when administered by mucosal routes. This is a particular problem with oral delivery; where high doses of antigen are required to generate even modest immune responses. In contrast, nasal delivery of antigens with a range of adjuvants or delivery systems has been shown to generate relatively potent immune responses and to protect against infection in animal models. Advances in immunology have demonstrated that a variety of cellular and humoral immune effector mechanisms, that are regulated by distinct Th1 and Th2 subtypes of T cells, mediate protection against different infectious diseases. The identification of adjuvants and immunomodulators, that can promote the selective induction of these distinct populations of T cells, has now made it possible to rationally design safe and effective mucosal vaccines against a range of infectious diseases of man. © 2001 Elsevier Science B.V. All rights reserved.

Keywords: Adjuvant; Vaccine delivery system; Immunomodulator; Infectious pathogen; Th1/Th2 cell; Antibody; Innate immunity; Acquired immunity

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1. Introduction

Traditional vaccines based on killed or attenuated bacteria and viruses have been responsible for preventing millions of deaths each year from infectious diseases. However, increases in antibiotic-resistant bacteria, the emergence of new pathogens and concerns over the safety of killed and attenuated vaccines have highlighted the requirement for new vaccines and for the refinement of existing vaccines to render them safer and more effective. Recent advances in our understanding of immunity to infectious diseases and in the identification of protective antigens on a range of pathogens, together with developments in gene cloning and expression, have enabled a more rational approach to the development of safer subunit vaccines, consisting of specific antigenic components of the pathogen. Furthermore, advances in our understanding of the immunological mechanisms of action of adjuvants, including those that are effective for mucosal delivery, have made it possible to design antigen–adjuvant combinations capable of inducing protective immunity against a range of infectious diseases.

Since the majority of human pathogens initially infect through the mucosal tissues, nasal or oral delivery of vaccines may facilitate the prevention of certain infectious diseases through the induction of local immune responses. In addition, mucosal immunization abrogates the need for injections and may reduce certain of the adverse reactions seen when the vaccines are administered by parenteral routes. Mucosal defense against pathogens consists of both innate barriers, such as epithelium and mucus, innate immune responses and adaptive immune responses. The innate immune response not only provides the first line of defense against infection, but also determines the nature of the acquired immune response. The acquired immune system, involving both humoral and cell-mediated immunity, usually develops after infection is established and while it can help in preventing infiltration of patho-

gens and assist in their elimination, it is most effective at preventing re-infection through the recall of immunological memory. The induction of a potent memory T and B cell response is the basis of successful vaccination.

2. Cell mediated and humoral immunity

Mucosal delivery of vaccines has the advantage over conventional parenteral immunization of stimulating both mucosal and systemic immune responses. Mucosal antibodies, secretory IgA in particular, may provide an early defense against invading pathogens, while serum IgG may be required for prevention of systemic infection, but also functions at mucosal surfaces by transudation from the serum [1]. Secretory IgA is induced when antigens contact mucosal surfaces and are transported to mucosal lymphoid tissue. Processing of the antigens leads to homing of specific IgA producing cells to various mucosal effector sites. Secretory IgA may protect against pathogens that replicate on or enter via mucosal surfaces by blocking their attachment or colonization and by neutralizing surface acting toxins. In addition, intraepithelial IgA may inhibit intracellular virus replication [1].

Cellular immunity, mediated by CD4⁺ and CD8⁺ T cells, also plays a major role in protection against infectious pathogens [2]. CD4⁺ and CD8⁺ T cells respond to antigens processed and presented by antigen presenting cells (APC) by distinct mechanisms. CD4⁺ T cells recognize exogenous antigen in association with major histocompatibility complex (MHC) class II molecules while CD8⁺ cytotoxic T lymphocytes (CTL) recognize endogenous antigen in association with MHC class I molecules. Therefore, to generate an effective CTL response an exogenous antigen has to be directed to the endogenous pathway of processing, for presentation to class I restricted T cells. CTL represent an important immune effector mechanism for clearance of many intracellular vi-

ruses, bacteria and parasites. Following recognition of processed viral and bacterial fragments presented on the infected cell surface, CTL destroy the infected cells. In addition, as CTL target all viral gene products that are expressed during viral replication, specific CTL responses may help to lower the viral load by interfering with viral assembly [1]. CD4⁺ T helper cells, on the other hand, provide help for B cells in antibody production, but also function in cell mediated immunity as producers of cytokines that are involved in a variety of immunoregulatory functions or have a direct effect on invading pathogens.

3. Th1 and Th2 cells

The CD4⁺ T cell population may be divided into further subpopulations on the basis of their function and the cytokines they secrete [3]. Products of bacteria and viruses, including lipopolysaccharide (LPS), bacterial DNA and dsRNA, activate cells of the innate immune system, including macrophages and dendritic cells, to produce IL-12 or IL-18 and drive the differentiation of naïve precursor Th cells towards the Th1 subtype [4]. Th1 cells secrete IFN- γ and TNF β and mediate cell-mediated immunity, such as delayed-type hypersensitivity, macrophage activation and inflammatory responses. However, Th1 cells can also provide helper function for specific IgG subclasses, particularly those involved in opsonization and virus neutralization [5]. Precursor Th cells are differentiated into Th2-type cells by dendritic cells matured in the presence of products from helminth pathogens and allergens under the influence of IL-4. Th2 induction is considered to be the default pathway and in the absence of microbial products that stimulate IL-12 production, antigens that lack immunomodulatory activity, such as many soluble recombinant proteins, will induce Th2 cells. Type 2 T cells secrete IL-4, IL-5, IL-10 and IL-13 and support IgG1 subclass and IgE production. Either Th1 or Th2 cells or a combination of both may be important in supporting antigen-specific secretory IgA responses. Th2 type responses have been shown to be important in the terminal differentiation of B cells [3], while the Th1 cytokine, IFN- γ , has been implicated in the induction of the

polymeric immunoglobulin receptor required for transport of secretory IgA [6].

Protective immunity against certain diseases is dependent on the differential induction of Th1 or Th2 responses. It has been shown that for several intracellular pathogens, protective immunity requires the development of Th1 responses and pathology may develop if Th2 cytokines are induced. While the induction of Th1 responses is desirable for immunization against many intracellular pathogens, Th2 responses may be more important in protection against pathogens such as *Helicobacter pylori* and helminth parasites and in the control of immune-mediated diseases, such as organ-specific autoimmune disorders and Crohn's disease. The pattern and interaction of cytokines induced following immunization is therefore critical in determining the outcome of an infection. The Th1 and Th2 subpopulations of CD4⁺ T cells are reciprocally regulated by a range of cytokines produced by the T cells themselves or by cells of the innate immune system [3,4]. Once an immune response is induced it tends to get polarized to one subtype, which is influenced by the APC and the cytokine environment at the site of immunization.

4. Role of adjuvants in stimulating innate cells that direct distinct arms of the acquired immune response

Nasally delivered subunit vaccines are often poorly immunogenic because they are highly purified and therefore do not contain the bacterial or viral components, which often function as built-in adjuvants in traditional vaccines. These components provide the danger signals for activation of innate cells, such as dendritic cells, the maturation of which is required for the induction of specific subtypes of antigen-specific T cells. Therefore adjuvants and delivery systems, for nasally delivered DNA and subunit vaccines, may be used not only to augment the strength of the immune response, but also to influence the types of cellular and antibody responses induced. The following sections of this article reviews recent advances in the nasal delivery of vaccines where immune responses can be manipu-

lated to the desired subtype as part of vaccine design strategy.

4.1. Microparticles

Microparticles, prepared from the biodegradable polymer poly(lactide-co-glycolide) (PLG), may act as efficient delivery systems for entrapped protein or polysaccharide antigens, viruses, plasmid DNA or peptides. The potential of PLG microparticles as a delivery system relies on their capacity to release entrapped antigen over extended periods and on their ability to deliver antigen directly to phagocytic APC. Following mucosal administration, microparticles are taken up by M cells and allow exogenous antigen to gain access to the endogenous route of processing for presentation to class I-restricted T cells [7]. Furthermore entrapment in PLG allows the CD4⁺ T cell responses induced with soluble protein antigens to switch from a predominant Th2 to a Th1 subtype [7,8]. Microparticles may therefore be important mucosal adjuvants for the induction of cellular immune responses to intracellular pathogens. Furthermore, humoral immune responses, in particular the persistence of serum antibody responses, is enhanced by encapsulation of vaccines in PLG. The size of the particles also appears to affect the outcome of the immune response, with the larger microparticles more effective for inducing cellular immunity and smaller nanoparticles favouring the induction of antibody responses [8].

It has been demonstrated that entrapment of antigens from HIV or from *Bordetella pertussis* in biodegradable microparticles is an effective means of inducing antibody and Th1 responses, and in the case of pertussis antigens protection against respiratory infection [8]. It has also been shown that intragastric administration of encapsulated peptides representing measles virus CTL epitopes may effectively prime systemic CTL responses in vivo [9]. Although to date, microparticles have generally been used for oral delivery of vaccines, they also have potential for nasal delivery of antigens, through activation of nasal associated lymphoid tissue and draining lymph nodes [10]. Protection from pneumonic plague was obtained after encapsulating recombinant subunit antigens from the intracellular pathogen, *Yersinia*

pestis, in PLG microspheres and administering intranasally to mice [11].

4.2. Chitosan

Chitosan, a cationic polysaccharide derived by deacetylation of chitin, has been shown to have potential as a delivery system for protein vaccines delivered by the nasal route. Chitosan appears to augment immune responses by enhancing the uptake of antigen across the nasal mucosa. However, this molecule can also boost T cell and antibody responses to co-injected foreign antigens delivered by the parenteral route, suggesting that it may also have adjuvant activity [12]. Chitin and its derivatives have been shown to activate peritoneal macrophages [13] and to stimulate the production of cytokines such as IL-1 β and TNF- α by cells of the innate immune system in vitro [14]. It has been shown that nasal delivery of filamentous haemagglutinin from *Bordetella pertussis* with chitosan may enhance local and systemic antibody responses [15]. Intranasal immunization with a mutant of diphtheria toxin formulated with chitosan enhanced neutralizing antibodies and Th2 responses in mice. The T cell responses observed following parenteral delivery of the same formulation were of a mixed Th1–Th2 subtype [12], suggesting that the route of delivery as well as the delivery system or adjuvant may influence the type of systemic immune response generated.

4.3. Monophosphoryl lipid A (MPL)

MPL, a derivative of lipopolysaccharide (LPS), primarily from *Salmonella minnesota*, which retains much of the immunostimulatory properties of LPS without the inherent toxicity, has been used extensively as an adjuvant for parenteral administration of antigens. The adjuvant activity of MPL is primarily attributed to its ability to activate APC and elicit cytokine production, in particular IL-12 by dendritic cells and macrophages, resulting in the induction of antigen-specific cellular immunity and enhancement of complement fixing antibodies [16]. Intranasal vaccination with MPL may be particularly beneficial in instances where both systemic Th1 type responses and local secretory IgA are desired, as may be

required for the elimination of many viruses and intracellular bacteria. Intranasal immunization with influenza virus antigens formulated with MPL induced antigen-specific IgA, CTL responses and antibody responses suggestive of Th1 type immunity following intranasal immunization [17]. While MPL alone can augment humoral and cellular immune responses to foreign antigens, it has been found to be more effective when combined with other adjuvants or delivery systems. For example, MPL with QS21, a non-toxic derivative of saponin, was found to significantly enhance Th1 responses to HIV gp120 recombinant glycoprotein [16]. MPL has also been shown to be effective as an adjuvant for augmentation of mucosal and systemic immune responses to intranasally delivered liposome-formulated *Streptococcus mutans* crude glucosyltransferase [18].

4.4. Live vector vaccines

Live vector vaccines, consisting of attenuated strains of bacteria or viruses, that express foreign antigens of unrelated pathogens, have been shown to be effective mucosal delivery systems. Due to their inherent adjuvant activity, live attenuated microbial vectors are highly immunogenic, and have potential for the expression of many unrelated foreign antigens. The use of live attenuated microorganisms as vectors has not always been very successful at generating antibody responses to the foreign insert, but because they undergo limited replication at target sites of mucosal immunization, live attenuated vectors provide an effective means of inducing CD8⁺ T cell responses. Attenuated *Salmonella* spp. are particularly attractive as potential live vectors because of their potent immunogenicity and their ability to deliver antigens via mucosal surfaces. Intranasal immunization with attenuated *Salmonella* spp. vectors carrying a plasmid encoding fragment C of tetanus toxin has been shown to generate high proliferative T cell responses against the foreign antigen [19]. However, safety concerns and environmental considerations still negates the use of many mucosally delivered vector candidates such as *Salmonella* spp. and, therefore, commensal lactobacilli have begun to receive attention as safe, non-invasive vectors. Nasal delivery of fragment C of tetanus toxin, expressed as a surface-exposed protein on

Lactobacillus plantarum, was shown to induce secretory IgA antibody responses and antigen-specific T cell activation in draining lymph nodes [20]. A new approach to live vector vaccine has been the insertion of antigens from human viruses or bacteria into plant viruses. Nasal or oral administration of chimeric plant virus particles induced potent mucosal and systemic immune responses to a fibronectin-binding protein of *Staphylococcus aureus* [21].

4.5. Lipid vesicles

Liposomes, or bilayered phospholipid membrane vesicles, have the capacity to adjuvant entrapped, surface-linked or admixed antigens. Antigen-specific IgG and local IgA responses have been shown to be enhanced after intranasal administration of inactivated measles virus with liposomes [22] and following nasal delivery of BSA-associated liposomes [23] in mice. Formulation of *Streptococcus mutans* antigen into liposomes has also been shown to enhance local secretory IgA responses following intranasal delivery in humans [24]. Virosomal influenza vaccine, comprising antigens from influenza virus incorporated in the membranes of phosphatidylcholine-containing liposomes, administered nasally with *Escherichia coli* heat-labile toxin (LT) as adjuvant, enhanced virus-specific IgA responses in human volunteers [25].

Formulation of antigen into liposomes may also induce antigen-specific cellular immune responses, including CD4⁺ Th1 cells and CD8⁺ CTL. Liposome-formulated *Yersinia pestis* vaccine induced strong IFN- γ responses, with no IL-4 detected, after intranasal administration in mice [26]. Liposomes encapsulating OVA were shown to elicit IFN- γ secretion, which was considerably stronger using negatively charged liposomes compared to neutral liposomes [27].

Immunostimulatory fractions extracted from the bark of the tree *Quillaja saponica*, called quill A or saponin, have been incorporated into lipid vesicles to generate another class of lipid-based adjuvant termed immune stimulating complexes (ISCOMs). ISCOMs have been shown to enhance local and systemic antibody responses to nasally administered respiratory syncytial virus envelope antigens [28]. Like liposomes, ISCOMs are also capable of promoting

the induction of CD8⁺ CTL and CD4⁺ Th1 cells. This appears to reflect the ability of these complexes to facilitate transport of antigen into the cytoplasm of APC for access to the endogenous route of processing and to stimulate IL-12 production from cells of the innate immune system [29].

4.6. Mucosal delivery of DNA

It was discovered in the early 1990s that direct intramuscular injection of plasmid DNA encoding an antigen resulted in the induction of humoral and cellular immune responses and protective immunity against infectious diseases in animal models. DNA vaccines allow the inclusion of many antigens simultaneously and may include additional genes expressing cytokines or costimulatory molecules, permitting the manipulation of the immune responses generated. The endogenous expression of proteins, encoded by the DNA, results in a presentation of antigens which facilitates the generation of cellular immune responses. Nasal immunization of mice with naked plasmid DNA has been shown to induce antigen-specific IgA and cellular Th1 responses to the parasite *Cryptosporidium parvum* [30] and to induce T cell dependent protection against *Chlamydia pneumoniae* [31]. However, when naked plasmid is administered intranasally it may degrade to some extent due to the presence of nucleases, causing loss of gene expression and poor immunogenicity. In order to limit degradation and increase plasmid uptake at mucosal surfaces, various formulations have been assessed for mucosal delivery. Formulation of DNA vaccines into liposomes or bioadhesive polymers [32] or DNA–lipid complexes [33] significantly enhances the immune responses to the encoded antigens. Nasal administration of a noninfectious vaccine, encoding firefly luciferase, elicited antigen-specific IgA and MHC-restricted CTL responses after formulation of the DNA with cytofectins [34]. Co-administration of naked DNA, encoding β -galactosidase, with LT intranasally has also been shown to result in higher cellular responses compared with DNA alone [35].

4.7. CpG motifs of bacterial DNA

In addition to coding for antigen, bacterial plasmid DNA itself may constitute a very powerful adjuvant

for systemic and mucosal immune responses due to the presence of unmethylated CpG motifs, which are generally absent from mammalian DNA. These adjuvants are most often given in the form of synthetic oligodeoxynucleotides (CpG-ODN) that provide a broad adjuvant effect, including stimulation of B cells to proliferate and secrete immunoglobulin and direct activation of monocytes, macrophages and dendritic cells to secrete various cytokines and chemokines. CpG-ODN induce cytokines such as IL-12, TNF- α and IFN- γ , with little secretion of Th2 cytokines, and therefore may be considered as immunoadjuvants where a Th1 type response is desired. In contrast, non-CpG-ODN, which also has been shown to have adjuvant effects at mucosal sites, would appear to selectively induce Th2-like responses [36]. It has been shown that CpG DNA may augment humoral and cell-mediated systemic and mucosal responses to Hepatitis B surface antigen following intranasal administration to mice [37]. It was found that CpG and CT act synergistically to provide potent antibody responses, which were stronger than when either adjuvant was used alone [37]. Therefore, CpG-ODN are novel adjuvants, either alone or in combination with other mucosal adjuvants, for the induction of mucosal humoral and cellular Th1 responses to co-administered foreign antigens.

4.8. Cytokines

Soluble cytokines regulate many of the molecular and cellular events during an immune response, including the selective induction and differentiation of Th1 and Th2 cells, therefore recombinant cytokines have also been assessed as vaccine adjuvants. In theory, certain cytokines should be capable of selectively enhancing the induction of IgG, IgA, CTL, Th1 or Th2 responses at mucosal surfaces. Boyaka et al. [38] showed that mucosally delivered IL-6 and IL-12 enhanced antigen-specific serum antibody responses to co-administered protein antigen, but only IL-12 was able to trigger antigen-specific secretory IgA responses. The potent proinflammatory cytokines, IL-1 α and IL-1 β , were found to enhance antigen-specific serum IgG, vaginal IgG and IgA, systemic delayed-type hypersensitivity and T cell responses when administered intranasally with OVA or tetanus toxoid antigens [39]. Intranasal

administration of a DNA vaccine, encoding HIV antigens, together with an IL-2 expression plasmid induced a strong antigen-specific Th1 response [40]. IL-12 has also been shown to augment the Th1-type response when administered nasally with a tetanus toxoid and cholera toxin vaccine [41].

Mucosal delivery of certain cytokines is also being assessed for the treatment of Th1-mediated inflammatory responses. Intranasal delivery of DNA encoding IL-10 resulted in diminished delayed type hypersensitivity reactions in mice primed with herpes simplex virus [42]. Furthermore, combined nasal administration of autoantigen and IL-10 suppressed experimental allergic encephalomyelitis in rats [43].

4.9. Cholera toxin and *E. coli* heat-labile toxin

The most powerful mucosal adjuvants described to date are cholera toxin (CT) and *Escherichia coli* heat labile toxin (LT). The mucosal immune system is able to recognize these molecules as a threat and a short time after they make contact with a mucosal surface a powerful immune response is generated against them. This anti-toxin response is so potent that strong responses may also be stimulated against foreign bystander molecules that are present simultaneously at mucosal surfaces. CT and LT are composed of two subunits: an enzymatically active A subunit with ADP-ribosylase activity that is responsible for the toxicity and a B subunit that mediates binding of the molecules to their receptors, the ganglioside GM1, on epithelial target cells. Once the toxins are internalized by the cells, the A subunit ADP-ribosylates the α subunit of a GTP binding protein which regulates the activity of adenylate cyclase and causes intracellular accumulation of cAMP.

Although LT and CT have been shown to be effective mucosal adjuvants for the nasal delivery of numerous antigens [44–46], their clinical use may be restricted due to their toxicity. Therefore, attempts have been made to separate the toxic effects of these molecules from their adjuvant activity. One novel approach is represented by the genetic manipulation of these toxins to render them partially toxic or completely non-toxic, but still adjuvant active. Single amino acid replacements have been introduced by site-directed mutagenesis, in the enzymatically active cleft of the A subunit to generate mutants such as

LTK63, LTR72 and LTK7. These mutants have been shown to enhance local and systemic immune responses to a variety of antigens following intranasal immunization [47,48].

The GM1 binding activity of the B-subunit of LT and CT results in the activation of acidic sphingomyelinases and an associated increase in intracellular levels of ceramide, which may lead to the activation of NF κ B [49] and the transcription of a number of immune response genes, such as those that regulate IL-12 production and B7 expression. Alternatively, the ADP-ribosylase activity of the A subunit leads to the accumulation of cAMP [45,50], a potent immunomodulator, which inhibits the development of Th1 responses but has enhancing effects on Th2 cells. Therefore, the adjuvant effect of LT or CT may be derived from independent contributions of the A and B subunits.

LT and LTR72, but not LTK63, were shown to inhibit the production of the proinflammatory cytokines, IL-12 and TNF- α , in LPS-stimulated macrophages [51] and CT, but not CT-B, suppressed IL-12 and TNF- α production by dendritic cells and monocytes [52]. Therefore, LT and CT or their mutants may enhance acquired immunity by stimulating innate immune responses and this approach may be used for the selective stimulation of Th1 or Th2 cells. The non-toxic mutant, LTK63, has been shown to enhance Th1 and Th2 responses to nasally delivered co-administered pertussis antigens, whereas the partially toxic mutant, LTR72, enhanced Th2 responses at lower doses of adjuvant [51]. CT has been shown to augment Th2 responses to orally delivered antigen in mucosal associated lymphoid tissue [53] and to enable human dendritic cells to polarize precursor Th cells towards a Th2 phenotype [54]. However, the immune response to a foreign entity not only depends on the adjuvant but also on the dose and route of immunization and the biochemical composition and properties of the antigen itself.

Mutants of LT and CT have been shown to induce CTL responses following mucosal vaccination, and therefore may be important in immune responses to intracellular pathogens. Intranasal immunization with HIV gag p55 protein coadministered with LTK63 or LTR72 [55] or immunization with a HIV-1 Env CTL determinant and LTR(192G) [56] was capable of inducing systemic antigen-specific CTL responses.

5. Conclusions and future perspectives

Traditional vaccines have been designed using an empirical approach, with little knowledge of the protective antigens or correlates of immunity. Nevertheless, this approach was highly successful and killed and attenuated vaccines were responsible for eliminating or significantly reducing the incidence of a number of infectious diseases, including smallpox, polio, diphtheria, tetanus, pertussis, mumps, measles and rubella. However, the major obstacles to the continued use and development of new live virus or whole cell bacterial vaccines relate to safety concerns. Furthermore, the introduction of more and more paediatric vaccines has resulted in an unacceptably high number of injections for infants in the first year of life. The move to purified subunit protein and conjugated polysaccharide vaccines has overcome many of the safety issues, but because of a reliance on alum as the adjuvant these have often not been very immunogenic and are still being delivered by parenteral routes.

Recent developments in genetic engineering together with significant advances in our understanding of protective immune responses has facilitated a more rational approach to vaccine design, based on recombinant protein or nucleic acid approaches. Furthermore, advances in the development of novel adjuvant and delivery systems has helped to identify vaccine formulations that are safe and effective when delivered by mucosal routes. The identification of immunomodulators that can direct the immune responses to humoral or cell mediated immunity and

that can selectively induce type 1 or type 2 T cell responses (Table 1), has now made it possible to design protective vaccines based on prior knowledge of the protective mechanism. For example, diseases like diphtheria and tetanus, which are largely mediated by bacterial toxins, can be prevented by toxin-neutralizing antibodies. Therefore, new generation vaccines against these diseases should be possible using genetically detoxified recombinant toxins delivered parenterally or nasally with a delivery system or adjuvant, such as chitosan, AB toxins or PLG nanoparticles, that can enhance antibody and Th2 responses. More effective acellular vaccines against pertussis may be possible by replacing alum with adjuvants or delivery systems, such as PLG microparticles, LT or CT mutants or IL-12, that can stimulate Th1 responses, which function together with antibody in the rapid elimination of the bacteria from the lungs. Finally, CD8⁺ CTL as well as CD4⁺ Th1 appear to be critical in the prevention and control of viral infections, including HIV and hepatitis C virus, and protective vaccines against these viral infections may be possible using delivery systems such as naked DNA, live vectors, IL-12 or immunomodulators that induce IL-12 production.

The use of delivery systems or immunomodulators that can be used to enhance immune responses to mucosally delivered vaccines should make it possible to design nasal or oral recombinant protein or DNA vaccines that can induce protective immune responses. The major obstacle to the development of non-replicating oral vaccines is the poor immunogenicity of antigens delivered by this route and

Table 1
Influence of adjuvant or delivery system on the induction of humoral or cell-mediated immunity to mucosally delivered vaccines

Adjuvant/delivery system	Humoral immunity	Cell-mediated immunity
Soluble	+ / –	–
Chitosan	++	–
CT/LT	+++	+
PLG nanoparticles	+++	++
QS21 + MPL	++	++
PLG microparticles	++	+++
LT mutants	++	+++
IL-12	++	+++
Live vectors	+	+++
Plasmid DNA/CpG	+	+++

Humoral and cellular immunity in arbitrary units represent the ability of adjuvants or delivery systems to enhance total serum IgG and Th2 responses or CTL and Th1 responses, respectively, to foreign antigens.

Table 2
Pros and cons of oral and nasal vaccines

	Oral	Nasal
Delivery	Easy	Simple device
Patient compliance	High	Moderate
Antigen digestion	Extensive	Limited
Antigen uptake	Poor	Good
Immunogenicity	Poor	Modest–good
Immunological tolerance	Possible	Possible
Antigen dose required	High	Medium
Adjuvant required	Powerful mucosal	Mucosal
Targeting required	Mandatory	Helpful
Safe	Yes	Probably

consequently the dose of antigen required (Table 2). However, the oral route ensures the greatest patient compliance and is considered to be the safest. Approaches to prevent degradation by encapsulation, to enhance uptake by targeting, and to improve immunogenicity by incorporating powerful mucosal adjuvants, such as CT or LT mutants, may bring further breakthroughs in oral vaccination. In the meantime the focus has turned to nasal vaccines, which are now showing considerable promise. A range of antigens with a variety of adjuvants or delivery systems have been shown to be immunogenic by the nasal route and protection has been demonstrated in a number of pre-clinical models of disease. Clinical trials using nasally delivered vaccines have been performed with influenza virus glycoproteins with LT [25,57], diphtheria and tetanus toxoids with polysorbate and glycerides [58], live attenuated respiratory syncytial virus [59], group B meningococcal vesicles [60], CRM197 conjugated pneumococcal oligosaccharide [61], liposome-formulated antigen from *Streptococcus mutans* [24] and killed whole cell pertussis alone [62]. Most of the trials have reported good immunogenicity and none have reported major adverse reactions. However, a recent study in mice has suggested that formulation of vaccines with CT may help to direct nasally delivered antigens to neural tissue via the olfactory bulb [64]. Parenterally delivered vaccines can also result in trafficking of vaccine components or inflammatory mediators to the brain and have been shown to induce inflammatory cytokines, which trigger biochemical and neurophysiological changes in mice [63]. Therefore, the balance between potent

protective immunity and controlled inflammation and reactogenicity, in particular adverse neuroimmunological responses, will need to be carefully monitored in the development of new and improved vaccines against infectious diseases based on nasal administration.

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