

NEW DEVELOPMENTS IN ORAL VACCINES AND MUCOSAL ADJUVANTS

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ABSTRACT

Mucosal immunity is the first line of defence of the organism against several pathogens and, at the same time, it is of critical importance in allergic diseases. Oral vaccines have been developed with the aim of enhancing the immune response to pathogens and for the treatment of allergic diseases. One of the major issues concerning oral vaccines is the use of oral adjuvants which could facilitate antigen presentation with the consequent induction of an effective immune response. The present review consists of an analysis, point by point, of the different patents that have been presented in the last 12 months in the different agencies: European (EP), US, and World Intellectual Property Organization (WIPO) and a general analysis of the future developments and trends in this emerging area.

Keywords: Adjuvant, allergic diseases, immunoglobulin, mucosa, mucosal immunity, oral vaccines.

INTRODUCTION

Infectious diseases are mostly acquired through mucosal surfaces. Mucosal immunity functions as the first defence barrier against infectious agents, preventing their attachment to epithelial cells by secretory immunoglobulin A (sIgA). Successful parenteral immunization induces systemic immunity but it does not regularly produce mucosal immune responses. On the contrary, antigen presented on mucosa cells trigger local, and at times distant, mucosal responses and systemic responses. Intramuscular (IM) and intradermal (ID) routes are the usual routes of vaccine administration in order to generate a systemic immune response. Once the pathogen, or the antigen, is available to be expressed by the antigen presenting cells, systemic immune responses are generated. However, the mucosa surface is larger than the

skin, approximately 200 times, and is the port of entry of many pathogens and allergens. Thus, the development of mucosal vaccines is highly encouraged.

Mucosal vaccines may be used in a broader range of individuals originating a reduction in the rate of infections. The administration of mucosal vaccines is non invasive and can be administered as an inhaler/nebulizer, a nasal spray and/or nose drops, it is simple to administer and the risk of contamination is lower than traditional vaccines .

The key element in mucosal immunity is the local production of sIgA antibodies. More than 80% of the antibodies present in the mucosa are IgA and its production, transport and regulation differs from the systemic immunoglobulins. IgA-mediated mucosal defence is located at different levels: (1) the lumen, where sIgA prevents pathogen adhesion and infection, blocks bacteria toxins and enzymes; (2) epithelial cells, where dimeric IgA can bind to intracellular antigen; and (3) the lamina propia, where dimeric IgA can complex with an antigen. Once the immune complex is formed, it is transported to the lumen .

Many vaccines which are currently being developed contain recombinant (peptides or polypeptides), or synthetic antigens, which are safer than attenuated, or inactivated pathogens. These recombinant and synthetic antigens require strong adjuvants to induce specific responses, since they are poorly immunogenic *per se*. Several adjuvants have been developed, which are stronger than aluminium salts, for parental administration, including TLR4 agonists (MPL) and oil-water emulsions (MF59, AS03). However, strong adjuvants have generally a high level of toxicity limiting their use for human vaccine formulations . Table 1 shows a list of adjuvants approved for use in humans by the FDA .

Adjuvant Licensed)	Name	(Year	Adjuvant class	Components
Alum (1924)			Mineral salts	Aluminium phosphate or aluminium hydroxide
MF59 (1997)			Oil-in-water emulsion	Squalene, polysorbate 80, sorbitan trioleate
AS03 (2009)			Oil-in-water emulsion	Squalene, Tween 80, α -tocopherol
Virosomes (2000)			Liposomes	Lipids. Hemagglutinin
AS04 (2005)			Alum-absorbed TLR4 agonist	Aluminium hydroxide, MPL

Table 1. List of adjuvants approved for human use.

In absence of a proper adjuvant, most current mucosal vaccines contain live-attenuated organisms. Up to now, mucosal delivery of subunit vaccines has not been successful. but different mucosal adjuvants have been developed. In animal models, cholera toxin (CT), derived from *Vibrio cholera*, is frequently used as mucosal adjuvant. The 84 kDa polymeric toxin consists of two subunits. The pentameric B subunit binds monosialotetrahexosylganglioside (GM1) gangliosides at the surface of eukaryotic cells and facilitates the insertion of the monomeric A subunit into the cytosol . CT is able to: 1) improve antigen presentation by macrophages, epithelial cells and B cells, 2) induces B cell differentiation and isotype switching,

3) it can selectively activate Th2-type CD4+ T cells while inhibiting Th1 type cells proliferation and cytokine production and/or it can activate both Th1 and Th2-type CD4+ T cells . The reasons for the contrasting effects could be due either to the route of administration, or to the nature of the antigen. A similar structure/function relationship is encountered with the *E coli* heat labile enterotoxin (LT) . Immune response to non immunogenic antigens can be achieved with LT when the adjuvant is mixed, or coupled to the antigen . Both, CT and LT are toxic in humans. These toxins, modified by site directed mutagenesis, seem to retain adjuvant properties while decreasing its toxicity in animal models . In summary, there is a need for adjuvants that are safe while retaining effectiveness for mucosal vaccines.

Classification of adjuvants

The new developments in mucosal vaccine adjuvants have been classified in this review as shown in Table 2.

1. Active immunostimulants

1.a. Biological

1.a.1. Whole bacterial cells as immune modulator (EP1534330)

The development is based on the finding that a whole cell of a bacterium from the genera *Rhodococcus*, *Gordonia*, *Dietzia* and *Tsukamurella* administered to a subject can elicit cellular immune activation . Although it has been suggested that individual components of bacterial cells could be used to elicit an adjuvant effect, by using the whole cell from those bacteria, a greater and longer lasting adjuvant effect is achieved, as compared with the response elicited by the administration of an individual component of the bacterium.

1.a.2. Compositions and methods for treatment of mucosal infections (US8637051)

Novel compositions and vaccines have been developed for prophylactic and/or therapeutic treatments of mucosal infections . The compositions of the present patent are intended to be administered orally and may be combined with known pharmaceutically acceptable carriers, solvents and excipients. A mucosal administrable composition, comprising one, or more antigens derived from at least one microorganism (virus or fungus), capable of causing infection, and an adjuvant able to induce Th1 cellular immune response is proposed. The adjuvant used in the compositions is a microorganism, or a part thereof, which is not an organism that is capable of causing infection at a mucosal surface and which can induce a Th1 cellular immune response. Ideally, the adjuvant should be a bacterium, such as *Mycobacterium*, or *Bifidobacterium* species. The bacteria *Lactobacillus acidophilus*, *Lactobacillus fermentum*, or *Mycobacterium vaccae*, or parts thereof, are preferred since they are capable of inducing a strong Th1 cellular response. They may be used live (*L. acidophilus* and *L. fermentum*), or as an inactivated preparation, as long as they are capable of inducing a Th1 response. It is considered that other bacteria would also be suitable as adjuvants, such as *L. casei*, *L. plantarum*, *L. rhamnosus* and *Bifidobacterium breve*. Recent work has shown that both live and

heat-killed *Lactobacillus casei*, associated with pneumococcal antigen (P-Ag) are effective mucosal adjuvants at the nasopharynx level, inducing IgA and IgG anti-P-Ag Abs in the upper and lower respiratory tract .

1.a.3. Packaging of immunostimulatory substances into virus-like particles: method of preparation and use (US8691209)

Virus like particles (VLPs) can be loaded with DNA oligonucleotides containing non-methylated cytosine triphosphate deoxynucleotide (C) and guanine triphosphate deoxynucleotide G (CpGs) . These CpG-VLPs generate significantly higher immunogenic response as compared to their CpG-free counterparts. Moreover, CpG-VLPs were able to increase B and T cell responses. The Th1 response response to the coupled antigens is similar to that produced to VLP itself.. In 2008, a study demonstrated that the immune response against hepatitis B antigen greatly increases in HIV patients when CpG is administered with the vaccine . Antigens attached to CpG loaded VLPs might be used for vaccination against tumors, chronic viral diseases or allergies.

1.a.4. Mucosal immunization to prevent prion infection (US8685718)

The mucosal route is effective for vaccines against prion diseases, since they are able to induce a good humoral immune response . The vaccines are composed of a prion protein, a fragment of the prion protein, or a non-amyloidogenic prion protein homolog, mixed with an adjuvant, and used to elicit humoral immune response after mucosal administration. As aforementioned, cholera toxin subunit B is a suitable adjuvant for this vaccine. The construct includes a vector encoding a prion protein, the fragment, or the homolog in an attenuated *Salmonella* host. The use of an attenuated *Salmonella* vaccine strain expressing the prion protein conferred protection against prion infection. Active and/or passive immunomodulatory approaches can also be developed that specifically target the scrapie prion protein, or other disorders. These new developments may have a significant impact on age-associated dementias.

1.a.5. Mucosal meningococcal vaccines (EP1587538)

The design involves: (1) a capsular saccharide antigen from serogroup C of *N. meningitidis* conjugated to carrier proteins, or a capsular saccharide antigen from serogroups A, C, W135 and Y of *N. meningitidis* and (2) a mucosal adjuvant, tri-alkylated chitosan . The invention also provides an immunogenic composition used for mucosal delivery, and a mucosal adjuvant. The capsular saccharides may be conjugated to carrier protein(s) in the possible compositions of the invention in order to provide the necessary support for inducing a good response to the saccharide moieties involved.

1.a.6. Compositions and methods for stimulating an immune response against infectious agents (EP1221970)

The patent involves compounds and approaches to stimulate immune responses to infectious agents .

Oral immunization induces strong cellular immune, sIgA and significant serum antibody responses . Moreover, oral immunization has been shown to maintain memory B-cell, a critical factor in the immune response against viral challenges . In order to generate a strong immune response, an effective mucosal adjuvant, usually an enterotoxin, must be included . Moreover, the antigen that has mucosal binding, mucoadhesive, lectin, or receptor-binding properties, or could be modified chemically to enhance mucosal binding, has been shown to induce good immune responses . Taking into account these properties oral vaccines should be constructed with these characteristics.

Membrane hemagglutinins (HA) are glycoproteins which mediate viral Influenza virus cell attachment and envelope fusion. Due to its structure, HA bind neuraminic acid rich glycoproteins, while LT-R72 and LT-K63 bind GM1-ganglioside, as well as galactose containing glycoproteins and lipopolysaccharides, all of which are found in the gut . Del Giudice *et al.* report intragastric administration of antigens with LT-K63 . However, as mentioned before, CT and LT adjuvants, which would enhance this response, cannot be used in humans due to its toxicity . The present invention contains influenza virus hemagglutinin and a LT-K63 or LT-R72 mutant of *E. coli* heat labile toxin in a pharmaceutically acceptable carrier for oral use.

1.a.7. Immunomodulatory compositions (US8557859)

Isolated immunomodulatory (e.g. immunostimulatory) polyhydroxylated pyrrolizidine alkaloids, like casuarine, are useful in therapy and prophylaxis (Fig. 1). The compounds have been claimed to: 1) increase the Th1:Th2 response ratio, 2) induce haemal restoration, 3) alleviate immunosuppression, 4) induce cytokine stimulation, 5) inhibit proliferative disorders, 5) stimulate innate immune response, 6) boost endogenous NK cell activity and 7) favour responses to vaccine . This invention refers to the use of casuarine and certain analogues as immunomodulatory (immunostimulatory or immunosuppressive) drugs. Casuarine can be isolated from several botanical sources, including the bark of *Casuarina equisetifolia* (Casuarinaceae), the leaves and bark of *Eugenia jambolana* (Myrtaceae) and *Syzygium guineense* (Myrtaceae) . Future studies should ascertain the role of these compounds in oral vaccine therapy.

1.a.8. Novel adjuvant (EP2221062)

Hemozoin is a hydrophobic hem polymer which is the detoxification product of the hem molecules found in the food vacuole of *Plasmodium* protozoa, and it can be produced by digestion of host haemoglobin by *Plasmodium* protozoa. Like CpG DNA, hemozoin acts as a ligand of Toll-like receptor 9 (TLR9). TLR9 is involved in the innate immune response to various pathogens including *Plasmodium*. The immune system is activated in a MyD88-dependent manner when TLR9 recognizes a ligand. The hemozoin synthesized from haemin chloride is called β -haematin. It is reported that hemozoin activates, *in vitro*, spleen cells and dendritic cells of mice. It is also reported that hemozoin has an adjuvant effect on the antibody production of ribonuclease A in mice. In addition, it is reported that β -haematin has an effect as an adjuvant of DNA vaccines and it is further reported that β -haematin functions as a ligand other than TLR9 DNA molecules. However, there was no disclosure that hemozoin and β -haematin could be used as a vaccine adjuvant for potentiating *in vivo* effect of allergen, bacterial, or viral vaccines. A combination use of the vaccine adjuvant composition of the present patent comprising hemozoin or β -haematin with a mucosal vaccine can induce mucosal IgA antibody production in the mucosal membrane, and can prevent, or effectively treat, the infections. The effect of β -haematin as mucosa adjuvant was demonstrated measuring IgG1 and IgG2a in serum and IgA in bronchial and intestinal washings in mice immunized intranasally.

1.a.9. Method of inducing mucosal immune responses to antigen with *Dioscorea* polysaccharides adjuvant (US8628809)

A method refers the use of *Dioscorea* polysaccharides as an adjuvants. The inventors show that the oral intake of *Dioscorea* polysaccharides produces an enhancement of antibody response to pneumococcal antigens (Pneumovax 23[®]) and ovalbumin administered orally. IgA responses to both antigens are increased in intestinal and pulmonary lavages. Because of the immunomodulatory effect of the *Dioscorea* polysaccharides, the vaccine composition is capable of overpassing immunological tolerance. In a previous published study, selected plant polysaccharides and phytochemicals were evaluated for use as a DNA vaccine adjuvant in a murine melanoma model. It was reported that a specific ethanol extract fraction (DsCE-I) from the tuber of the plant *Dioscorea* enhanced the protection against melanoma after immunization with a gene-based vaccine. Another study evaluated specific *Dioscorea* phytoextracts for *ex vivo* use as a bone-marrow-derived dendritic cell-based vaccine adjuvant for cancer immunotherapy. Fractionated *Dioscorea* extracts (DsII) were assayed for their effect on maturation and functions of dendritic cells (DC) *ex vivo*. The phytoextract [(50-75% ethanol-precipitated fraction of *Dioscorea alata* var. *purpurea* (DsII-TN5)], stimulated the expression of CD40, CD80, CD86, and IL-1 β in DCs

and down regulated the expression of TGF- β 1 and, consequently, could decrease the generation of tolerogenic T cells.

1.a.10. Therapy-enhancing glucan (US8791252)

The patent involves the use of an effective amount of glucan in order to enhance antibody and vaccine efficacy . Cheung claims that “oral beta-glucans derived from barley, or oats can greatly enhance the anti-tumour activity of anti-tumour monoclonal antibodies in xenograft models”. A glucan purified from barley, in combination with the antibody 3F8 was able to reduce tumour growth in mice, an effect that was not observed when glucan was used alone.

Further studies should be performed with oral β -glucan in order to ascertain its possible role in cancer therapy.

1.a.11. Glucan-based vaccines (US8679506)

Glucans, glucose-containing polysaccharides, are widely distributed in fungi and other microorganisms. The glucan may be presented on the surface of a protease-treated microbial cell or may be presented as a protein-glucan conjugate. There are 2 general structures depending on the glucose link: 1) α -glucans, found in several organisms, i.e. *Streptococcus mutans* cell wall contains α -1,3- and α -1,6-glucans, and 2) β -glucans, β -1,6-glucans, which occur frequently in fungi . In a fungal cell wall, β -1, 3-glucan microfibrils are combined with chitin microfibrils to form the inner skeletal layer which are linked to the outer layer that consists of β -1,6-glucan and lipomannan rich proteins. For example, 50-70% of the cell wall of *C. albicans*, is composed of β -1,3- and β -1,6- glucans. The full length native β -glucans are branched and insoluble. Fungal glucans are poor immunogens and have not previously been considered for eliciting protection. Nonetheless, anti-glucan-antibodies show broad spectrum microbicidal activity.

De Smet and co-workers evaluated the potential use of β -glucan particles (GP) as oral antigen delivery system and adjuvants. “GP are efficiently internalized by human intestinal epithelial cell lines (Caco-2 and HT-29 cells), without exerting negative effects on cell viability. GP triggered the expression of pro-inflammatory cytokines IL-23p19, IL-8 and the β -glucan receptors dectin-1 and TLR2 by activated Caco-2 cells, and CCL20 in HT-29 cells. In contrast, the expression level of TGF- β was significantly down regulated in HT-29 cells”. The oral administration of GP-OVA induced “OVA-specific IgA, secretory-IgA and secretory component production in intestinal fluids. GP vehicles were able to deliver OVA via an oral route allowing efficient antigen presentation along with adaptive immune activation, resulting in a Th17-biased response and the production of OVA-specific IgA, secretory-IgA and secretory component antibodies”.

The invention describes an immunogenic composition comprising a glucan and a pharmaceutically acceptable carrier. The glucan may be substituted by a glucan mimotope, a peptidomimetic of a glucan mimotope, or nucleic acid encoding a mimotope. β -glucans containing one, or more β -1,6 linkages are preferred. The glucan used in accordance with the invention comprises a mixed glucan. The glucan mixture has a ratio of β -linkages to α linkages of 2:1, or higher. The authors claimed that when the mixed glucan is administered to a mammalian species, the composition elicits protective anti-glucan antibodies . More studies are required to show the importance of these antibodies as protectors for a pathogen interaction with the mucosa.

1.b. Synthetic

1.b.1. Stimulation of vaccination by angiotensin peptides (WO2014116587)

The present design provides angiotensin peptide compositions and methods for use in vaccination . The inventors have demonstrated that angiotensin peptides are broadly applicable as vaccine adjuvants, and could have a potential use in vaccination. The pharmaceutical compositions of the invention may include any suitable angiotensin peptide. The inventors found high levels of sIgA anti-gp120 in oral and vagina secretions upon either intramuscular vaccination, or topical application of Fel-0-Vax FIV (feline immunodeficiency virus vaccine) with angiotensin peptides, but not in their absence. The ATRQ β -001 vaccine comprised a human angiotensin II receptor type 1 derived peptide (ATR-001) conjugated with Q β bacteriophage virus-like particles. This vaccine was assayed in animal models of hypertension . The vaccine is able to generate antibodies that decrease the signal transduction induced by angiotensin II without affecting the binding of the hormone to its receptor. The decrease in blood pressure is important and may influence new developments in the field.

1.b.2. Methods and products for inducing mucosal immunity (US8574599)

Immunostimulatory oligonucleotides containing a CpG motif have been used in several vaccine constructs. In particular this patent refers to the use of CpG for inducing mucosa immunity . The CpG mixture may be administered alone, or in combination with antigen and/or with other adjuvants. The authors claim that an effective amount of the compound administered to a mucosal surface induced mucosal immune responses. The formula of the compound is "5'X₁X₂CGX₃X₄ 3'", wherein C and G are unmethylated, X₁, X₂, X₃, and X₄ are nucleotides and an antigen to induce the mucosal immune response". Interestingly, the antigen is not encoded in a nucleic acid vector which makes it relevant for future developments.

1.b.3. Immunostimulatory oligonucleotides (EP2759306)

The present patent also uses immunostimulatory oligonucleotides to induce an antigen-specific immune response . Since bacterial DNA (unmethylated CpG) activates B and natural killer cells , synthetic oligodeoxynucleotides (ODN), containing CpG, are safer and simple stimuli for immune cells motifs. “These immune stimulatory effects of native phosphodiester backbone CpG ODN are highly CpG specific since the effects are dramatically reduced if the CpG motif is methylated, or otherwise eliminated, or altered” . It has been previously reported that immunostimulatory activity of CpG oligonucleotides is dependent on the number of CpG motifs, the sequences flanking the CpG dinucleotide, the location of the CpG motif(s) and the spacing between the CpG motifs . An immunostimulatory oligonucleotide having the 3' CpG motif removed is presented that retains its immunostimulatory activity. Authors of the present vaccine propose the use of an immunostimulatory oligonucleotide comprising the nucleotide sequence 5' TCGTCGTTTTTCGGTGCTTTT 3'. There are different embodiment possibilities: the immunostimulatory oligonucleotide comprises one or more modified linkages. The immunostimulatory oligonucleotide comprises one or more phosphorothioate linkage and all internucleotide linkages of the oligonucleotide are phosphorothioate linkages. The immunostimulatory oligonucleotide comprises at least one lipophilic substituted nucleotide analogue and a pyrimidine-purine dinucleotide. A vaccine comprising an antigen and an immunostimulatory oligonucleotide comprising the nucleotide sequence aforementioned is able to induce a Th1 immune response.

1.b.4. Adjuvanted glucans (EP2227248)

The patent focused on the possible treatment of fungal infections . The construct comprises a glucan containing β -1,3-linkages and/or β -1,6-linkages and conjugated to a carrier protein directly, or via a linker, one or more sequences of at least five adjacent non-terminal residues linked to other residues by β -1,3-linkages . The carrier protein of the invention can be a bacterial toxin or toxoid, or a mutant thereof. The inventors demonstrate that single molecular glucans may be more immunogenic than polydisperse glucans, particularly when the composition also comprises an adjuvant. This structure can be obtained by chemical synthesis which is similar to the natural glucan.

1.b.5. Compositions and methods for inducing an immune response (US8658603)

Recent evidence indicates that two subsets of T lymphocytes, regulatory T (Treg) cells and IL-17-producing T helper (Th17), are playing reciprocal roles in immune responses. Treg cells act as suppressors of the immune response against tumours and infectious pathogens . The present invention provides compositions comprising immunostimulatory ligands (ISL) based on amino acid sequences (peptides), or cyclic peptides generated using a backbone cyclization, and methods of inducing an immune response in a subject. In the preferred

embodiments, an ISL comprises the motif Q/K-K/R-R-A-A (SEQ ID N°: 1) (e.g., QKRAA (SEQ ID N°:2), QRRRAA (SEQ ID N°:3), KKRAA (SEQ ID N°:4) or KRRRAA (SEQ ID N°:5)) or Q/R-K/R-R-A-A (SEQ ID N°:6) (e.g., RKRAA (SEQ ID N°: 13) and RRRRAA (SEQ ID N°: 14)). Authors present a method of inhibiting T cell tolerance in a subject consisting of the administration of an effective dose of a mixture containing an isolated cyclic peptide comprising an amino acid sequence selected from the aforementioned group SEQ ID NOS.: 1-6, 13 and 14 under conditions where T cell tolerance is reduced in the subject. Experiments, conducted to determine the effect of isopentenyl-pyrophosphate (IPP) on the activity of indoleamine 2-3 dioxygenase (IDO), concluded that this compound had a very strong synergistic effect with IFN- γ on IDO activation .

1.b.6. Antigen-and-drug vehicle comprising synthetic peptide, and mucosal vaccines using the same (EP2281574)

The innovation consists of generating an antigen-and-drug (AD) vehicle available for nasal, transmucosal, and transdermal administration, that could also be used as nasal/mucosal vaccine . The AD vehicle is a complex of pulmonary surfactant protein B and/or pulmonary surfactant protein C and a lipid(s). In addition, a mucosal vaccine is constricted using the AD vehicle and an antigen. Kido and co-workers [67] claimed that “the selective production of IgA antibodies and the production of both IgA and IgG antibodies are convertible by adjusting the weight ratio V/A between the AD vehicle amount (V) and the antigen amount (A)”. Trying to build an effective mixture, the authors found “peptides that are smaller in size, but have a stronger antibody-production-inducing, or enhancing effect”. In particular, the peptides are “effective in inducing the production of secretory IgA antibodies, or the production of both secretory IgA and serum IgG antibodies. Phospholipid(s) are usable, preferable including phosphatidylcholine, dipalmitoylphosphatidylcholine, phosphatidylserine, and phosphatidylglycerol. Examples of usable fatty acids include lauryl acid, myristic acid, palmitic acid, stearic acid, palmitoleic acid, and oleic acid”. In 2011, Mizuno *et al.* reported that “Surfacten®, a bovine pulmonary surfactant free of antigenic c-type lectins, is a useful mucosal adjuvant for nasal vaccination” .

1.b.7. Methods of using a vaccine composition containing synthetic adjuvant (US8609114B2)

Reed described a synthetic homogeneous compound with adjuvant properties, glucopyranosyl lipid adjuvant (GLA). The authors claim that “synthetic GLA offers a consistent vaccine component from batch to batch without the fluctuations in contaminants, or activity that compromised natural-product adjuvants”. Thus, the invention is directed to compositions and methods that advantageously employ the synthetic glucopyranosyl lipid adjuvant (GLA) as an adjuvant and vaccine component. “Other embodiments include “a vaccine composition comprising (a) an antigen; a glucopyranosyl lipid adjuvant (GLA) and a

toll-like receptor (TLR) agonist. In further embodiments, the TLR agonist is selected from lipopolysaccharide, peptidoglycan, polyI: C, CpG, 3M003, flagellin, *Leishmania* homolog of eukaryotic ribosomal elongation and initiation factor 4a and an imidazoquinoline immune response modifier". In case of a co-adjuvant, this is selected from the group consisting of "alum, a cytokine, a detergent, and a block copolymer or biodegradable polymer". Moreover, the carrier, when needed, is selected from the group consisting of calcium phosphate, oil-in-water emulsion, water-in-oil emulsion, liposomes, and microparticles .

1.b.8. Vaccine Composition (EP2647387A1)

The present invention relates to a sublingually administrable vaccine composition useful to be a preventive or therapeutic agent for infectious diseases . The vaccine contains a toll-like receptor 4 (TLR4) agonist, a toll-like receptor 2/6 (TLR2/6) agonist, and cyclic dinucleotide, or a derivative. This composition is capable of effectively inducing a systemic immune response and a mucosal immune response when is sublingually administered as an adjuvant together with an antigen derived from an infectious disease.

2. Antigen delivery systems

2.a. Emulsions

2.a.1. Antigen-adjuvant compositions and methods (US8790658)

Authors present vitreous compositions of an antigen and adjuvant . In one example, the vitreous compositions are in the form of foam. In other example, the antigens are proteins or peptides. In another example, the adjuvants are aluminium salt adjuvants. In other example, the vitreous compositions contain polyols and/or synthetic polymers that can form a glass.

2.a.2. Immunogenic compositions comprising nanoemulsions (WO2014052971)

Barker and co-workers designed the stimulation of immune responses using nanoemulsions compositions and methods of administration . Nanoemulsions could be effective in clinical therapeutic and preventative medicine (e.g., vaccination) and research applications.

2.a.3. Adjuvant and vaccine compositions (WO2013138334)

The patent involves the preparation of adjuvants for vaccines that includes lecithin, polymer and one, or more, additives . The polymer is polyacrylic acid-based and the additive is one or more of a glycoside molecule and/or a sterol. In order to prepare the mixture, lecithin has to be hydrated and then a polymer, dissolved in saline solution or water, is mixed with the hydrated lecithin to form the adjuvant. Additives can be included prior to, or after, hydration of the lecithin and polymer to form the construct for vaccine delivery.

2.a.4. Novel mucosal adjuvants and delivery systems (WO2014070709)

Adjuvants comprising chitosan cross-linked with an aldehyde or mannosylated chitosan are provided in this composition . The adjuvant-antigen combinations can be used in vaccine formulations and the vaccine formulations can be used to vaccinate animals against the source of the antigen, or to enhance the immune response in a subject.

2.a.5. Microemulsions with adsorbed macromolecules and microparticles (US8734832)

The present formulation comprises microparticles with adsorbent surfaces . The microparticles are “a polymer, such as a poly (a-hydroxy acid), a polyhydroxy butyric acid, a polycaprolactone, a poly orthoester, a polyanhydride, and are formed using cationic, anionic, or non-ionic detergents. The surface of the microparticles efficiently adsorbs biologically active macromolecules, such as DNA, polypeptides, antigens, and adjuvants”. The authors described “an oil droplet emulsion having degradable oil and an emulsifying agent”. The authors also claim that the mixture comprises “immunogenic compositions having an immunostimulating amount of an antigenic substance, and an immunostimulating amount of an adjuvant composition” in order to produce an effective and durable immune response.

2.b. Microparticles and nanoparticles

2.b.1. Lipophilic microparticles containing a protein drug or antigen (EP1071407B1)

The solid, lipophilic microparticle of the present invention comprises a lipophilic substance and an active ingredient . The microparticle of the present invention further comprises hyaluronic acid, or an inorganic salt thereof. Representative inorganic salts of hyaluronic acid include sodium hyaluronate, potassium hyaluronate, ammonium hyaluronate, calcium hyaluronate, magnesium hyaluronate, zinc hyaluronate and cobalt hyaluronate. The microparticle thus prepared has an average particle size ranging from 0.1 to 200 nm, preferably from 1 to 50 nm, more preferably 1 to 10 nm. The microparticle containing an active ingredient has several advantages: (1) the active ingredient is not denatured and retains its full activity; (2) it releases the active ingredient fully over a prolonged time; (3) it is easily dispersed in a lipophilic medium such as an oil, and the dispersion thus obtained has a low viscosity and retains the full activity of the active ingredient; (4) it is easily fused with the phospholipid membrane of the body to effectively transport the active ingredient into the body; (5) when it is orally administered, it can be translocated into M cells of Peyer's patches located in the small intestine.

2.b.2. Mucoadhesive nanoparticle delivery system (WO2013188979A1)

The present disclosure relates to a mucoadhesive nanoparticle delivery system . The nanoparticles are formed from amphiphilic macromolecules conjugated to a mucosal targeting moiety in such a manner that the surface of the nanoparticle is coated with the targeting moiety. The surface density of the targeting moiety can be tuned for adjustable targeting of the nanoparticles to a mucosal site without substantially compromising the stability of the particles. The particles were found to have high loading efficiency and sustained release properties at the mucosal site.

2.c. Tight Junction Agonists

2.c.1. Synthetic peptides that enhance tight junction permeability (US8557763B2)

The present invention provides methods for the treatment of disease by administering a composition comprising a peptide tight junction agonist in combination with a therapeutically effective amount of an active agent . The present invention provides novel peptides that enhance tight junction permeability and their use as therapeutic agents and their use in materials and methods to facilitate the delivery of therapeutic agents. In some embodiments, novel peptides that enhance tight junction permeability are used to facilitate the uptake of therapeutic agents across biological barriers comprising tight junctions. Such peptide tight junction agonists are used in compositions to modulate an immune response. Such peptide tight junction agonists are used in compositions to raise an immune response against an antigen. The peptides of the present invention are non-toxic, their effects are reversible, lack endotoxin contamination, readily synthesized and inexpensive to produce and purify.

2.c.2. Methods and compositions for enhanced delivery of macromolecules (US8697074B2)

The invention provides compositions and methods that enhance the delivery of large macromolecules (i.e., greater than 10 kDa), such as antigen-binding polypeptides, across tight junctions . Such methods and compositions are particularly useful for delivering therapeutic antigen-binding polypeptides to the central nervous system, via intranasal administration, for the treatment of neurological disorders.

3. Carriers

3.1. Drug transporter, and adjuvant and vaccine each utilizing same (US8580274B2)

The aim of the invention is to provide a drug delivery vehicle capable of allowing a vaccine, or adjuvant, to reach a target cell or tissue efficiently, while being capable of improving the immunogenicity of the vaccine or capable of enhancing the immunostimulating effect of the adjuvant, as well as a vaccine, or adjuvant . Said drug delivery vehicle contains a multimeric protein having a coiled coil structure and a ligand molecule to a receptor of an immune cell. This drug delivery system, which is specific to a cell and a tissue, has been

developed for preventing infectious diseases and establishing new therapeutic methods against cancer. In order to improve the immunogenicity of a component vaccine, a technology which utilizes a fusion protein of an antigen and a complement 4 binding protein (C4bp) is proposed. This technology had no ability of transporting an antigen site-specifically, although it allowed for association of the antigen. The present authors found that by allowing a vaccine, or adjuvant molecule, to be carried on a carrier formed by fusing a multimeric protein having a coiled coil structure in a compact state, with a ligand molecule to an immune response cell receptor in a design reducing steric hindrance, an efficient transportation to a target tissue or cell becomes possible while exhibiting an excellent immunopotentiating effect.

3.2. Glycosylceramide adjuvant for saccharide antigens (US8790654B2)

The invention provides compositions and kits comprising a saccharide antigen conjugated to a carrier; and an alphaglycosylceramide adjuvant . It has been found that suppression of anti-saccharide immune responses by alpha-glycosylceramides can be reversed by conjugating the saccharide to a carrier.

4. Mixed components and others

4.1. Method for generating dry vaccine powder formulation for intranasal delivery (EP2689785A1)

Mucosal vaccination can result in secretory sIgA responses in the respiratory tract and oropharyngeal region. One important characteristic of mucosal sIgA antibodies is that they can provide cross-protection against antigenically distinct viruses. Mucosal sIgA can provide protection against a viral strain that differs from the strain used to generate the vaccine. For example, influenza virus H1N1 can drift to H2N1, or H1N2. One of the major drawbacks of the influenza vaccines, formulated as liquids, is that it can chemical degrade and consequently become inactive. Moreover, these formulations can be sensitive to temperature. In order to prevent inactivation, liquid vaccines are stored and distributed in a temperature range between 2 and 8 degrees Celsius, increasing the cost of the vaccine. Thus, the generation of vaccines that are stable at room temperature would decrease costs and increase marketing. Dry powder vaccines may be a suitable solution. One approach is freeze-drying vaccines; however, the mixture could be difficult to dissolve and unreliable and, spray-freeze-drying (SFD) is costly.

The patent described methods for generating “a dry vaccine powder formulation which overcomes the limitations of previous freeze drying methods, resulting in high potency powdered vaccines with high flowability”. “One, or more antigens, such as a pathogen or a component thereof (e.g., a whole inactivated influenza virus) with one or more agents (e.g., a saccharide and/or buffer, e.g., phosphate buffer) can be used. A liquid vaccine formulation can be freeze-dried (e.g., comprising quick freezing in liquid nitrogen) to generate a powder (e.g., a vaccine powder). The powder can comprise fine particles and can be stable at room

temperature. After freeze drying, the powder can be blended with one or more excipients to form a dry vaccine powder formulation". This formulation could be very useful in providing vaccines in difficult to access areas and for underdeveloped countries, where due to the climatic conditions, the transport of vaccines and medications is difficult .

4.2. Compositions and methods for encapsulating vaccines for the oral vaccination and boosting of fish and other animals (US8778384B2)

The invention patent designed for oral delivery of a vaccine to animals, particularly aquatic animals, overcomes "the shortcomings of the encapsulation systems" . As described by the authors: "The mucoadhesive properties of the compositions of the present invention provide a successful method of transmucosal drug delivery, especially for lower vertebrates with less developed digestive systems and no Peyer's Patches, such as fish. One aspect of the present invention provides for a method of producing a bioadhesive delivery vehicle for vaccination of aquatic animals, wherein the delivery vehicle is in a form of dry microparticles comprising an immunogenic agent embedded or impregnated in a composite matrix of cross-linked chitosan, and at least one oligosaccharide, or short chain polysaccharide. Any applicable oligosaccharides, or short chain polysaccharides, may be used in the composition. Common short chain polysaccharides include maltodextrins and cyclodextrins. The oligosaccharides may include fructo-oligosaccharides (FOS), galacto-oligosaccharides (GOS) or inulin. Additionally, the dry microparticles include a beta glucan, and squalane". This mucoadhesive compounds can be interesting for vaccine delivery in several tissues.

4.3. Mucoadhesive drug delivery devices and methods of making and using thereof (US8529939B2)

The patent refers to mucoadhesive drug delivery comprising biocompatible purified proteins combined with biocompatible solvents and mucoadhesive agents. It may also include pharmacologically active agents. The drug delivery devices of the present invention adhere to mucosal tissues, providing a vehicle for delivery of the pharmacologically active agent(s). Preferred mucoadhesive proteins having enhanced mucoadhesive properties when included in the mucoadhesive devices are egg white proteins such as ovalbumin, and plant proteins such as soy protein. The present embodiments have shown to provide excellent adhesion (e.g. strength and contact duration) and have provided excellent transmucosal delivery of the pharmacologically active agents. The mucoadhesive delivery systems of the present invention are intended to incorporate drugs that may be delivered locally, or systemically. A mucoadhesive device may deliver an anaesthetic and/or analgesic agent to alleviate the pain associated with many oral mucosal wounds, or lesions. In other embodiment, allergens for desensitization such as house mite allergens can be delivered.

4.4 Improved adjuvant system for oral vaccine administration (WO2013151595)

The present invention relates to the construction of orally administrable immunogenic compounds by adsorbing an antigen and a C-type lectin (CTL)-agonist to an aluminum adjuvant, adding a polymer having pH dependent solubility to form a vaccine formulation and adding the vaccine formulation to a low pH solution to precipitate the polymer. Protection of the vaccine from degradation in the stomach may be achieved with polymers that protect the vaccine. An acrylic resin like Eudragit® that precipitates below pH 5.5 can be used for conservation. The CTL receptor ligand(s) comprise saccharides with a terminal end phosphate group, or phosphodiester backbone, to bind a metallic adjuvant such as an aluminum salt. This allows for receptor mediated endocytosis of co-localized antigen and adjuvant. The adjuvant stimulates the production of Th1 and Th2 cytokines as well as targeting the antigen to endosomes, where cross- presentation of antigen on MCH I and II molecules can occur. Experiments with these embodiments were able to increase the total serum IgG over one log compared to unadjuvanted antigen. This demonstrates a potential for antigen dose sparing through utilization of the adjuvant system.

CURRENT & FUTURE DEVELOPMENTS

There is an urgent need for new approaches for preventing infections. Mucosal vaccines targeting mucosal surfaces, the main entry of pathogens in our body, have the advantage to induce a local immune response at the site where most initial infections take place. However, in order to activate the mucosal immune system properly, mucosal vaccines have to take into account different issues in addition to the nature of the antigen used. These are related to the

mucosal site where the vaccine is delivery, the vehicle where the antigen is included or the adjuvant used. All these issues have been considered by the different inventions retrieved in this review. As can be noted, most of inventions have been aimed to increase the immune response, and therefore related to the adjuvant properties of novel substances suitable to be administered on mucosal surfaces. Many of these are ligands of TLR on dendritic cells, acting as immunomodulating agents on these initiators of immune response, although other substances have also been considered. Several of these inventions relate to activation of TLR9, that may modulate the immunity. For example it has been shown that CpG oligonucleotides may

induce human eosinophil survival either indirectly or directly and they cause airway hyperreactivity by themselves . Thus, one might hypothetise that one possible adverse problem with some of these inventions would be an increase in the occurrence of eosinophilic/allergic/ asthma-like syndromes.

For a mucosal vaccine, the target mucosa used as induction site (nasal, gastrointestinal, vaginal, etc) has strong implications to get the proper effectors' site responding to vaccination. In this sense, those vaccines intended to be administered orally may require antigen delivery systems with shielding properties, able to protect the active substance from gastrointestinal degradation. Some remarkably inventions are focused on this fact. Mucoadhesive properties of different substances and vehicles have also been disclosed. The goal is to maintain the antigen close to the mucosal surface long enough for a better release, and thus increasing its bioavailability. Mucosal vaccines are a hopeful approach for the treatment and prevention of infection diseases. However, mucosal immunity is quite complex and the immune response triggered by an antigen may result in a good effectors' response, or tolerance induction. In this sense, oral tolerance by mucosal administration of vaccines remains a challenge for mucosal vaccine development. As either response may be depending on a number of factors, that are being elucidated, it is not surprising that inventions derived from unexpected, or not obvious results, do appear. New developments to obtain better mucosal vaccines are warranted in the following years.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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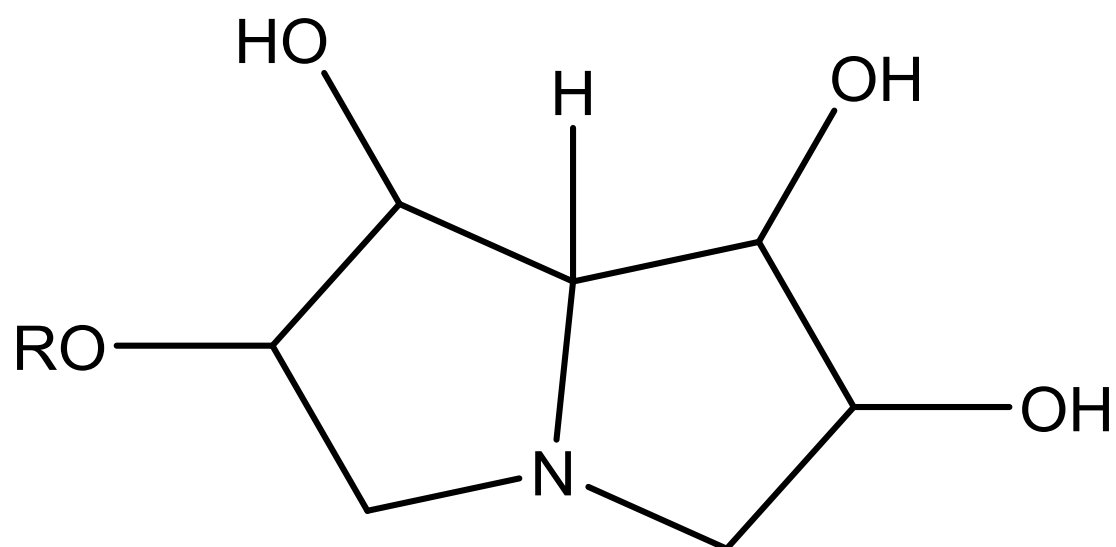


Figure 1. The structure represents a polyhydroxylated pyrrolizidine, a structure described as immunomodulatory.

Class	Type	Patent Reference
1. Active immunostimulants	a. Biological	EP1534330 US8637051 US8691209 US8685718 EP1587538 EP1221970 US8557859 EP2221062 US8628809 US8791252 US8679506
	b. Synthetic	WO2014116587 US8574599 EP2759306 EP2227248 US8658603 EP2281574 US8609114B2 EP2647387A1
2. Antigen delivery systems	a. Emulsions	US8790658 WO2014052971 WO2013138334 WO2014070709 US8734832
	b. Microparticles & nanoparticles	EP1071407B1 WO2013188979A1
	c. Tight Junction Agonists	US8557763B2 US8697074B2
3. Carriers		US8580274B2 US8790654B2
4. Mixed components and others		EP2689785A1 US8778384B2 US8529939B2 WO2013151595

Table 2. New developments in mucosal vaccine adjuvants