Journal of Entomology and Zoology Studies

Available online at www.entomoljournal.com

J E Z S Journal of Entomology and Zoology Studies

E-ISSN: 2320-7078
P-ISSN: 2349-6800
JEZS 2017; 5(2): 795-802

Received: 12-01-2017
Accepted: 13-02-2017

Shafiya Imtiaz Rafiqi
Division of Veterinary Parasitology, Indian Veterinary Research Institute, Izatnagar, Bareilly-243 122 Uttar Pradesh, India

Saroj Kumar
National Research Centre on Equines, Sirsa Road, Hisar-125001, Haryana, India

Asima Zehra
Division of Veterinary Public Health, GADVASU, Ludhiana, Punjab, India

Dinesh Kumar
Division of Animal Nutrition, Indian Veterinary Research Institute, Izatnagar, Bareilly-243 122 Uttar Pradesh, India.

Shikha Jain
National Research Centre on Equines, Sirsa Road, Hisar-125001, Haryana, India

Khushboo Sethi
National Research Centre on Equines, Sirsa Road, Hisar-125001, Haryana, India

Pavan Kumar Yadav
Faculty of Veterinary and Animal Sciences, IAS, Banaras Hindu University, Varanasi-221004, Uttar Pradesh, India

Correspondence
Pavan Kumar Yadav
Assistant Professor (Veterinary Biochemistry), Department of Physiology and Biochemistry, Faculty of Veterinary and Animal Sciences, IAS, Banaras Hindu University, Varanasi-221005 Uttar Pradesh, India

Nanovaccinology: Dawn of biomimetic vaccine carriers

Shafiya Imtiaz Rafiqi, Saroj Kumar, Asima Zehra, Dinesh Kumar, Shikha Jain, Khushboo Sethi and Pavan Kumar Yadav

Abstract
A wealth of genomic and proteomic information on microorganisms and parasites, together with recent advances in adjuvant and delivery systems, is being harnessed to develop nanovaccines against infectious and parasitic diseases. The use of nanoparticles in vaccine formulations allows not only improved antigen stability and immunogenicity but also targeted delivery and slow release. However, so far, nanoparticles have not proved capable of surmounting most of the barriers like toxicity issues, clearance from biological system, DNA instability and differences in expression systems. Nevertheless, advances in nanoparticle engineering and understanding of nanoparticle characteristics, is creating new opportunities for the development of nanovaccines as antigen delivery system. This Review focuses on recent progress in development of nanoparticle based antigen delivery vehicles, their use in different diseases, major bottlenecks and challenges to realizing the potential of nanoparticles.

Keywords: Adjuvant, nanoparticles, delivery vehicles for vaccine antigens

1. Introduction
The practice of immunization began with the variolation against small pox in China and India and since then, the whole living organism being used was reduced to mere nanoparticles or subunits [1]. Though the means of practicing immunization overwhelmingly changed as the insights into biotechnology and immunology grew, the basic concepts are same. Even today vaccination is meant to expose the host to a simplified molecular identity (antigen) along with a immunostimulant (adjuvant) that replaces the inherent immunostimulation of a pathogen's infection with a less dangerous sustained response [2]. One of the most important drawbacks of subunit vaccines and synthetic antigens is the quantum of immune response elicited is far less than live or attenuated vaccines [3]. Incorporation of novel adjuvants and/or use of alternative delivery systems have shown promise in augmenting the immunogenicity of subunit vaccines and controlling many dreaded diseases [4].
The purpose of vaccination with any formulation is to emulate the innate and adaptive responses of the immune system to infection [5]. Antigen-presenting cells particularly, dendritic cells serve as a link between the innate and adaptive immune responses [6]. Toll-like receptors (TLR) present on the surface of APCs act as pattern recognition receptors and thus act as "molecular guards". On recognition of a foreign PAMP (pathogen associated molecular patterns) by TLR plethora of cellular responses are initiated like maturation of antigen presenting cells, redistribution of MHC molecules from intracellular compartments to the cell surface, secretion of cytokines and chemokines and cytoskeleton reorganization [7].
A successful adjuvant needs to targets the antigen to antigen-presenting cells by formation of multimolecular aggregates, or by binding antigen to a cell-surface receptor on APCs and direct antigen presentation by MHC class I or MHC class II pathways while at the same time avoiding lasting damage to the host [8, 9]. There is no denial of the fact that conventional whole cell live or attenuated vaccines are generally better immunogens than subunit vaccines and to redress this challenge of molecular mimicry, next generation vaccines incorporating nanoparticles are the new ventures [10]. The use of nanotechnology in vaccinology, in particular, has been increasing exponentially in the pasr decade, leading to the birth of the "nanovaccinology" [11]. Since the impetus for use of nanoparticles kept growing, they have been as delivery vehicles or for improvisation of antigen processing as immunostimulant adjuvant [12].

Nanoparticles either enclose the antigen within itself or project it onto their surface. The beneficial effects of encapsulating the antigen are sustained life of short live or rapidly degrading antigens and increased outreach of the localized immune response [13]. Conjugation of antigens onto nanoparticles can allow presentation of the immunogen to the immune systems possibly in the similar way that it would be presented by the pathogen, thereby provoking a similar response [14]. Moreover the recent advances in biotechnology and designing have led to synthesis of nanoparticles that led to site directed delivery of antigens hence increasing specificity manifolds [15]. Also being explored is the potential for nanoparticles to deliver vaccines through non-traditional methods such as topical, inhalation, or optical delivery as well as combining several antigens to the same particle to realize the dream of one vaccine for many diseases [16]. The power of nanoscale engineering can be vividly appreciated in the pathogen like particles that have emerged over years as tools of vaccine delivery. Different classes of pathogen like particles include synthetic particulate systems, virus-like particles and bacterial outer membrane vesicles.

Virus like particles (VLPs**)**

For the success of any nanoparticle based vaccine approach, the foremost and utmost important characteristics to be taken care of are the ease of production, cost effectiveness and ability to stimulate strong immune response. VLPs have been among the first studied nanoparticle based systems that are in concordance with the basic desired features of a vaccine delivery system. Typically in the size range of 20-150nm, VLPs consists of a self-assembled viral envelope, generated from a single protein to form a multimeric complex displaying a high density of epitopes [17]. VLPs are non-replicating and non-infectious particles that can neither integrate into host genome nor undergo any recombination with host or any defective virus. This has tremendous impact on the safety value of VLPs being used as delivery agents. VLPs can be engineered to express additional proteins either by fusing these proteins to the particle or by expressing multiple antigens. Using this approach, VLPs can be generated which provide protection not only against the virus of origin but also against heterologous antigens. Moreover, polysaccharides antigens or haptens can be chemically coupled onto the viral surface to produce bioconjugate VLPs [18]. Vaccine formulations based of VLP technique have already come in market. First VLP-based Recobivax HB was approved in 1986 against hepatitis B. Recently, two more VLP-based vaccines against HPV have been approved in United States, Cervarix in 2009 and Gardasil in 2006 [19]. Many others VLP-based vaccines are approved for veterinary use or in clinical trials, demonstrating the versatility of VLPs and their future in vaccine development. GlaxoSmithK- line's malaria vaccine called Mosquirix, the antigen is delivered via hepatitis B capsid VLP. Phase 3 trials have yielded encouraging results and provided protection against the parasite [20].

Biocompatible particulate based vaccines

Subunit vaccines are poorly immunogenic as compared to live or attenuated vaccines. The very basic idea of pathogen like particles is to mimic the physiochemical characteristics of a natural pathogen avoiding the deleterious effect on the host but enhancing antigen delivery to the immune system. Pathogen like particles (PLP) carriers can be formulated using nanoparticles obtained from polylactic acid (PLA) and poly lactic-co-glycolic acid (PLGA), that are safe and biocompatible, liposomes and even simple lipid emulsions [21, 22]. Antigens are then either incorporated inside the core or onto the surface of these particles. The meticulous ability to target the antigen presenting cells (APCs) and generate co-stimulatory signals renders the particulate antigens as one of best antigen delivery vehicles [23]. Furthermore by altering the physiochemical characters of the synthetic particles they can cater special needs of different vaccine formulations.

Liposomes

Liposomes are self-assembling particles consisting of a phospholipid bilayer shell with an aqueous core [24]. Based on the design of phospholipid bilayers, liposomes can be unilameller or multilameller, consisting of single or multiple concentric lipid shells separated by water layers. As a consequence, liposomes can be tailored to incorporate both hydrophilic antigens into the aqueous core as well as hydrophobic antigens within the phospholipid bilayers. Although preparation of liposomes is highly sophisticated procedure it can be summarized explaining the reverse phase evaporation process. Typically most of the methods are based on a reverse phase evaporation process in which an organic solvent such as chloroform or methanol is used for dissolving phospholipids (such as monophosphoryl lipid A or phosphatidylcholine). Water is then added, along with the antigen, and the solvent is evaporated resulting in large unilameller vesicles [25]. Alternatively, liposomes can form in water by introducing a high energy input such as sonication, or nitrogen gas under high pressure. The another method for preparing unilameller vesicles without subjection antigens to high energy, which can sometimes be destructive, is by dissolving lipids in a detergent with a high critical micelle concentration, such as octylglucoside. The solution then dialyzed against a buffer containing the antigen which results in the formation of liposomes. In any of these methods, cholesterol can be added to provide additional stability. Various other approaches to encapsulate antigens inside liposomes include repeat freeze thaw cycles [25], a pH gradient, or an ammonium sulphate method with antigen encapsulation rates varying between 25 and 72% [26].

Polymeric nanoparticles

A portfolio of synthetic polymers are used to prepare nanoparticles, *viz*. poly d, l-lactide-co-glycolide (PLG), poly d, l-lactic-coglycolic acid (PLGA), poly g-glutamic acid (g-PGA), polyethylene glycol (PEG), and polystyrene [27]. These polymeric nanoparticles entrap antigen for delivery to certain cells or sustain antigen release by virtue of their slow biodegradation rate. PLGA has been used to carry antigen derived from various pathogens including *Plasmodium vivax* with mono-phosphoryl lipid A as adjuvant, hepatitis B virus (HBV), *Bacillus anthracis*, and model antigens such as ovalbumin and tetanus toxoid [28].

The most commonly used poly α-hydroxy acids for preparing polymeric nanoparticles are either PLGA or PLA which are often synthesized using a double emulsion-solvent evaporation technique [29]. Firstly, to obtain a primary emulsion, a polymer of choice is dissolved in an organic solvent like ethyl acetate, or methylene chloride followed by the addition of the antigen, which is then vortexed. The addition of emulsifying agents like polyvinyl alcohol or polyvinyl pyrrolidine, to this primary emusion forms water-in-oil-in-water emulsion. This results in the polymer precipitating around the antigen. The solution is then left to

allow solvent evaporation and then dried to prevent degradation of the polymer due to water- catalyzed ester hydrolysis [30]. The use of this method is limited since antigen entrapment efficiency is low and there is a possibility of protein denaturation at the oil-water interface. The addition of stabilizers such as surfactants or sugars, including trehalose and sucrose, provide stability against denaturation by keeping the protein hydrated in its native state. An alternative method for ensuring stability of protein makes use of poly (aminoacids) such as poly(γ-glutamicacid) (γ-PGA), poly(ε-lysine), poly(L-arginine), or poly(L-histidine) which do not require an emulsion step in their synthesis. These amphiphilic copolymers self-assemble via hydrophobic interactions to form polymeric structures consisting of a hydrophobic core and a hydrophilic outer shell. Moreover, γ-linked glutamic acids in γ-PGA are not easily recognized by common proteases resulting in added stability.

Natural polymers based on polysaccharide have also been used to prepare nanoparticle adjuvants, such as alginate, pullulan, inulin, and chitosan. In particular, chitosan based nanoparticles have been widely studied due to physico-chemical properties viz. biodegradability, nontoxic nature and ability to be easily modified into desired shapes and sizes. These nanoparticles have been used in various vaccines including HBV vaccines, Newcastle disease vaccines and DNA vaccines. Inulin, a well-known activator of complement via the alternative pathway, serves as an efficient adjuvant. Nanoparticle adjuvants developed from inulin, such as Advax™, have shown enhancement of immune response in vaccines against various viruses including influenza and hepatitis B [31]. Chitosan nanogels have been frequently used in antigen delivery, such as *Clostridium botulinum* type-A neurotoxin subunit antigen Hc for an adjuvant free intranasal vaccine, and recombinant NcPDI antigen for *Neospora caninum* vaccination [32].

Inorganic nanoparticles

Apart from biodegradable materials, non-degradable nanoparticles are also being investigated for vaccine delivery [33]. Gold, carbon, and silica are widely used as nanodelivery vehicles which either encapsulate the antigen or covalently attach it to the surface. The uniformity in the shape and size is the most essential factor for maintaining antigen loading consistency. Generally 2-50nm size range is used however using chloroauric acid as the starting solution; the gold is reduced to forms spherical particles of either 10-20nm or 2nm in diameter depending on whether a mild or strong reducing agent is used. Other types of gold nanoparticles have been used as carriers for antigens derived from other viruses such as Influenza and Foot and Mouth disease, or as a DNA vaccine adjuvant for human immunodeficiency virus (HIV) [34]. Carbon nanoparticles are another commonly studied composition for drug and vaccine delivery. They are known for their good biocompatibility and can be synthesized into a variety of nanotubes and mesoporous spheres. The diameter of carbon nanotubes (CNTs) used as carriers is generally 0.8-2 nm with a length of 100-1000 nm, while the size of mesoporous carbon spheres is around 500 nm. Multiple copies of protein and peptide antigens can be conjugated on to CNTs for delivery and have enhanced the level of IgG response. Mesoporous carbon nanoparticles have been studied for application as an oral vaccine adjuvant [35]. One of the most promising inorganic materials for nanoparticle based delivery system is silica. Silica-based nanoparticles (SiNPs) are biocompatible and have excellent properties as nanocarriers for various applications, such as selective tumor targeting [36], real-time multimodal imaging, and vaccine delivery. The abundant surface silanol groups are beneficial in terms of introducing additional functionality, such as cell recognition, absorption of specific biomolecules, improvement of interaction with cells, and enhancement of cellular uptake [37, 38]. Calcium phosphate nanoparticles can be created by mixing calcium chloride, sodium citrate and dibasic sodium phosphate under specific conditions. They are non-toxic and can be formed into a size of 50-100 nm. These nanoparticles are useful adjuvants for DNA vaccines and mucosal immunity, and show excellent biocompatibility.

ISCOMS

Colloidal saponin cage like particles containing micelles of around 40nm can be used as self-adjuvanting vaccine delivery systems and are collectively known as ISCOMs. Two types of ISCOMs have been described, both of which consist of cholesterol, phospholipid (typically either phosphatidyl ethanol amine or phosphatidylcholine) and saponin (most often QuilA from the tree *Quillaia saponaria*) [39]. Classically, ISCOMs have been used to entrap viral envelope proteins such as from Herpes simplex virus type1, Hepatitis, and Influenza [40-42]. However, proteins from a range of bacteria and parasites including *Escherichia coli*, *Brucella aborus,* and *Plasmodium falciparum* have also been used to assemble ISCOMs. Complexes without viral proteins are also used and are often referred to as ISCOM matrices. ISCOMs are self-assembling at an optimal ratio of 1:1:5 (phospholipid: cholesterol: saponin) for matrices or 1:1:5:0.1/1 for classical ISCOM forming in the presence of a non-ionic detergent, which is then removed using dialysis or ultracentrifugation. The resulting complex is a pentagonal dodecahedron arrangement of micelles containing saponin and lipid held together by hydrophobic interactions and stabilized through its negative surface charge [43].

Nano sized emulsions

Over the years, oil-in-water or water-in-oil types have emerged as excellent adjuvants for vaccine delivery. The droplet size varies from 50 nm to 600 nm, carrying antigens inside their core for efficient vaccine delivery or simply mixed with the antigen. One commonly used emulsion is MF59TM, an oil-in-water emulsion which has been licensed as a safe and potent vaccine adjuvant in over 20 countries [44]. It has been widely studied for use in influenza vaccines. Another is Montanide™, a large family of both oil-in water and water-in-oil emulsions, including ISA 50 V, 51, 201, 206 and 720. Montanide ISA 51 and 720 have been used in Malaria vaccines [45]. Montanide ISA 201 and 206 have been used in foot-and-mouth disease vaccines.

Self-assembling protein systems

Self-assembling proteins can be tailored to act like virus like particle and ensemble the viral or bacterial protein unto itself. The antigen is genetically fused with the self-assembling protein and self-assembly leads either to packaging of protein inside the vault or projection on the surface of self-assembling protein. Ferritin is a protein that can self-assemble into nearly-spherical 10 nm structure. It has emerged as nanoparticle based vaccine carrier for influenza virus haemagglutinin (HA) [46]. Vault nanoparticles have been used to enclose the major outer membrane protein of *Chlamydia muridarum* for studies of mucosal immunity [47].

Outer membrane vesicles

Outer membrane vesicles (OMVs), first report has come almost 50 years ago, are naturally occurring proteoliposomes that obtained from Gram-negative bacteria. These vesicles are 50-250 nm condensing electron dense material, corresponding to bacterial periplasm, inside a single lipid bilayer. The growing consensus about OMVs containing a variety of immunoactive virulence factors, led researchers to exploit this potential of OMVs in use in vaccines [48]. OMVs were found to elicit protective humoral and mucosal immune responses, independent of adjuvants or pathogenic components, against the bacterium from which they were isolated. Apart from their ability to stimulate an effective immune response, OMVs have additional advantages that make them viable vaccine candidates.

a) Like synthetic nanoparticle vaccines, they are classified as acellular, making them attractive replacements for inactivated pathogen or live attenuated vaccines.
b) OMVs are able to present protein antigens in their native conformations, which is hypothesized to be important for effective antibody production.
c) Bacterial vesicles have been shown to be stable after long-term storage at 58°C, which is an essential aspect of commercial viability.

It is generally accepted that protective antibodies are generated against one or a few dominant antigens located on the vesicle membrane surface. Therefore, to explore the application of this vesicle against pathogenic bacteria, wild type OMVs were reengineered at the genetic and molecular level. The most crucial breakthrough in adapting OMVs into generalized PLP vaccines has come from their recent adaptation into heterologous antigen carriers. In this regard, the ability to remodel the outer surface of *Escherichia coli*-derived OMVs with a variety of recombinant antigens served as an important first step. Shortly thereafter, it was demonstrated that recombinant antigens displayed on engineered OMVs derived from hyper-vesiculating *E. coli* were capable of eliciting strong antibody titers in immunized mice [49].

The use of OMVs as vaccines is not without challenges. Lipopolysaccharide (LPS) being a major component of OMVs, is an area of concern considering the potential issue of residual endotoxicity. Even though research has shown that LPS in a membrane, such as is found in OMVs, is 100 times less toxic than purified LPS, there is still ample motivation to adapt OMV production protocols to further detoxify OMV LPS. For example, detergent extraction is a popular method for isolating OMVs because it decreases LPS content. Other methods involve structurally modifying the toxic lipid A to less harmful derivatives by genetically modifying the OMV producing host strain. Specific examples include introducing exogenous genes, such as pagL, or mutating/deleting endogenous genes, such as msbB.

Conjugation of nanoparticle and antigen: interphase interactions

Antigens can be delivered to the immune cells by nanoparticles as a part of the delivery system i.e. the co-ingestion of antigen and nanoparticle by the immune cell, or they can act as a transient delivery system, i.e. protecting the antigen and then release it at the desired location [50]. For nanoparticles to function as a delivery system, an association of antigen and nanoparticle is typically necessary. Over the years, nanoparticles based on silica, gold, and calcium phosphate, have been studied thoroughly for use as a delivery system and have thus been engineered to promote antigen attachment [51]. Attachment of antigen has been achieved through simple physical adsorption or more complex methods, such as chemical conjugation or encapsulation. Basic principle behind the adsorption of antigen onto a nanoparticle is generally charge or hydrophobic interaction [52]. Hence the interaction between nanoparticle and antigen being relatively weak, it may lead to *in vivo* rapid dissociation of antigen and nanoparticle. However, encapsulation and chemical conjugation offer stronger interaction between nanoparticle and antigen. In encapsulation, antigens are mixed with nanoparticle precursors during synthesis, resulting in the encapsulation of antigen, when the precursors particulate into a nanoparticle. The antigen is released only when the nanoparticle has been decomposed *in vivo* or inside the cell. On the other hand, for chemical conjugation, the antigen is chemically cross-linked to the surface of a nanoparticles [53]. The antigen is taken up by the cell together with the nanoparticle and is then released inside the cell. In soft matter nanoparticle delivery system, such as those based on ISCOM, VLPs, ISCOMATRIX™, or liposomes, binding of antigen is acquired through chemical conjugation, adsorption, encapsulation, or fusion at DNA level.

Interaction of nanoparticle with Antigen presenting cells (APC)

The effectiveness of nanoparticles to deliver the antigen to APC's is the centre of interest in commitment towards development of nanovaccines. As specialized APCs efficiently uptake and process antigen, dendritic cells (DCs) and macrophages are often targeted in vaccine design. The parameters that play significant role in antigen uptake are size, charge and shape of nanoparticles. Generally, nanoparticles having a comparable size to pathogens can be easily recognized and are taken up efficiently by APCs for induction of immune response. DCs preferentially uptake virus-sized particles (20-200 nm) while macrophages preferentially uptake larger particles (0.5-5 μm) [54]. Smaller particles (20-200 nm) get easy access to DCs residing in lymph nodes by exploiting interstitial circulation to access the lymphatic system, whereas larger particles (0.5-2 mm) remain at the injection site and are taken up by peripheral DCs and tissue-resident macrophages [21]. Moreover, there is a strong correlation between the mode of particle uptake and the size of the particles. Particles with smaller diameters (<200 nm) are taken up primarily via receptor-mediated endocytosis, while particles with larger diameters (>500 nm) are traditionally taken up by phagocytosis or macropinocytosis [55].

Surface charge is another important parameter that defines the role of nanoparticles in the activation of immune response. Cationic nanoparticles electrostatically interact with the anionic cell membranes, thereby induce higher APC uptake [56]. Liposomes and nanoscale lipid emulsions formed from cationic lipids (such as Novartis' MF591 and GlaxoSmithKline's AS031) have been shown to enhance immunity; the positively-charged liposomes more easily interact with the negatively charged cell surface of DCs [57]. Similar results have been observed by constructing nanoparticles using cationic polymers. More sophisticated approaches towards specifically targeting DCs encompass the conjugation of antibodies specific for certain externalized membrane targets, such as the endocytic receptor DEC205, to the surface of nanoparticles.

Particle shape also plays an equally important role in the interaction between nanoparticles and APCs. For big particles (>1μm), particle shape plays a dominant role in phagocytosis by macrophages as the uptake of particles is dependent on the local shape at the interface between particles and APCs [58]. Worm-like particles with high aspect ratios (>20) exhibited negligible phagocytosis compared to spherical particles [59]. On the other hand, spherical gold nanoparticles (AuNPs) (40 nm) were more effective in generating high antibody titer than other shapes (cube and rod) or reduced size, even though the rods (40 nm × 10 nm) were more efficient in APC uptake than the spherical and cubic AuNPs [60]. Hydrophobicity has been added as another parameter that affects immune response for example higher immune response was observed for hydrophobic particles than hydrophilic ones [61].

Maturation of vaccine-stimulated DCs, requires not only antigen presentation, but also the presence of co-stimulatory molecules. These molecules bind to pattern recognition receptors (such as Toll-like receptors), invoking DC activation and maturation when delivered in conjunction with the appropriate antigen [62]. Thus such components can be used to modify particle based vaccines in order to mimic stimulatory signals present on pathogens. Inclusion of these co-stimulatory molecules leads to activation of both cellular and humoral immune responses. TLRs are the well studied class of co-stimulatory receptors, and numerous synthetic TLR ligands have been identified and incorporated into nanoparticle vaccines. For example, CpG oligodeoxynucleo-tides (CpG-ODNs), which engage TLR-9, were shown to protect against West Nile encephalitis when co-delivered in a nanoparticle vector [62]. Similarly, poly I:C, a synthetic analogue of dsRNA, has been incorporated into nanoparticle-based vaccines as a TLR-3 agonist, mimicking viral pathogens. Lipid A, an endotoxic component of Gram-negative bacteria, has been incorporated into liposomes and serves as a strong adjuvant and TLR-4 binder [21].

Fate of nanoparticles inside biological system

The fate of the nanoparticles *in vivo* depends on the design of nanoparticles that inturn requires a thorough understanding of the interaction of nanoparticles with biological systems. Physicochemical properties of nanoparticles, including size, shape, surface charge and hydrophobicity influence the interaction of nanoparticles with plasma proteins and immune cells [64]. This interplay of physicochemical characteristics as well as morphology of vascular endothelial is crucial in the distribution of nanoparticles in various organs and tissues of the body.

The lymph node (LN) is an ideal organ for vaccine delivery since vital cells of the immune system, B and T cells, reside there. For eliciting an effective immune response it is mandatory that the antigen should reach the lymph node either by direct drainage or by migration of well-armed peripheral APCs [65]. Size of nanoparticles has a direct relation with its distribution to the LN. Like nanoparticles with a size range of 10-100 nm can penetrate the extracellular matrix easily and travel to the LNs where they are taken up by resident DCs for activation of immune response [66]. Particles of larger size (>100 nm) linger at the administration point and are subsequently endocytosed by local APCs, while smaller particles (<10 nm) get drained to the blood capillaries [67]. Moreover distribution of nanoparticles also depends on other parameters like the route of administration, biological environment to which nanoparticles are exposed and nanoparticle clearance from the body.

Design of nanoparticle vaccines also needs to consider nanoparticle sequestration from the body. Accumulation of nanoparticles in different organs and tissues can take place if nanoparticles are not degraded or excreted from the body. Renal clearance through the kidneys can excrete nanoparticles smaller than 8 nm [68]. Surface charge also plays an important role in determining renal clearance of nanoparticles. Many workers have documented that for same sized particles, ease of renal clearance follows the order of positively charged < neutral < negatively charged [69]. This may be attributed to the presence of negatively-charged membrane of glomerular capillary. On the other hand,earance though liver allows excretion of nanoparticle larger than 200 nm [70]. Surface charge also plays role in biliary clearance with an increase in surface charges showing increased distribution of nanoparticles in the liver. Furthermore, a study reported shape dependent distribution of nanoparticles where short rod nanoparticles were predominantly found in the liver, while long rods were found in the spleen. Short rod nanoparticles were excreted at a faster rate than longer ones [71].

Hurdles in the use of nanoparticles

The limitations of NPs for the delivery of vaccines range from concerns over the toxicity of the particles, to difficulties in producing the materials and presenting antigens in their native form.

1. The production of suitable NPs can have some technical challenges. For example, although insect cell lines are widely used to express VLPs, but they are unable to glycosylate proteins in the same way as mammalian cells [72].
2. One of the greatest obstacles with liposome delivery systems is their instability [73]. One of the ways by which this has been overcome is by modifying the surface with a hydrophilic polymer, such as glycol (e.g., glycol chitosan, polyethylene glycol).
3. The scale-up of production of sterile polymeric particles has also been problematic, though this has been overcome by scaled-up spray-drying techniques.
4. An ongoing concern with the introduction of NPs into biological systems has been their potential toxicity. As such the materials would be otherwise non toxic but conversion to nanoparticle form or chemical reactions during production or interactions inside the biological system may render them unsafe [74].
5. In its naturally occurring mineral state titanium dioxide is biologically inert, however, when administered as a NP smaller than 20 nm in diameter it causes an inflammatory reaction in animals and humans [75].
6. Gold is generally regarded as an inert material, safe, and is used routinely for medical implants, however, on the contrary gold NPs with a diameter of 1.4 nm act very distinctly and have been shown to penetrate cells and nuclear membranes and bind irreversibly in the major grooves of DNA causing instability [76].
7. Other toxicity concerns associated with NP is the accumulation within cells, particularly with continuous exposure or long- term use [77].

Scope of nanovaccines in parasitology

a) A prototypic malaria vaccine based on a highly versatile self-assembling polypeptide nanoparticle (SAPN) platform that can repetitively display a B cell immunodominant repeat epitope (DPPPPNPN) 2D of the malaria parasite *Plasmodium berghei* circumsporozoite

protein has been developed. SAPN platform not only functions to deliver an ordered repetitive array of B cell peptide epitopes but operates as a classical immunological carrier to provide cognate help to the P4c-Mal-specific B cells [78].

b) A candidate malaria antigen, VMP001, was conjugated to the lipid membrane of the nanoparticles, and an immunostimulatory molecule, monophosphoryl lipid A (MPLA), was incorporated into it, creating nanoparticle vaccine VMP001-NPs. Vaccination with VMP001-NPs promoted germinal center formation and elicited durable antigen-specific antibodies compared to vaccines composed of soluble protein mixed with MPLA. Antibodies raised by NP vaccinations also exhibited enhanced avidity and affinity towards the domains within the circumsporozoite protein implicated in protection and was able to agglutinate live *P. vivax* sporozoites. These results demonstrate that these VMP001-NPs are promising vaccines candidates that may elicit protective immunity against *P. vivax* sporozoites [79].

c) Cationic solid–lipid nanoparticle (cSLN) formulation was used to administer a DNA vaccine harbouring the *L. donovani* A2 antigen along with *Leishmania infantum* cysteine proteinases [CPA and CPB without its unusual C-terminal extension (CPB_CTE)] which led to induction of specific Th1 and Th2 clones, indicating a mixed immune response and the production of IFN-γ and IL-10, although IFN-γ was much higher than IL-10 [80].

d) The vaccination of mice with nanogel encapsulated recombinant NcPDI (recNcPDI) protein and challenge infection with *Neospora caninum* tachyzoites improved survival of intraperitoneal vaccinated mice [32].

e) Chitosan nanoparticles loaded with plasmid DNA encoding Rho1-GTPase protein of *Schistosoma mansoni*, showed that oral immunization was not able to induce high levels of specific antibodies but induced high levels of the modulatory cytokine IL-10. Mice immunized only with chitosan nanoparticles presented 47% of protection against parasite infection, suggesting an important role of chitosan in inducing a protective immune response against schistosomiasis [81].

f) Chickens immunized subcutaneously with an *Eimeria* recombinant profilin protein plus Montanide™ ISA 70 VG (ISA70) or Montanide™ ISA 71 VG (ISA 71) water-in-oil adjuvants, showed higher levels of transcripts encoding interferon-γ, interleukin (IL)-2, IL-10, and IL-17A [82].

Conclusion

Recent insights in the role of innate immune system, as a base for adaptive immune response to vaccines, have catered overwhelming support in favour of adjuvants. Adjuvants have been used for more than a century now to aid a strong immune response. The use of nanoparticles as vaccine carriers improves antigen stability and immunogenicity, along with targeted delivery and slow release of antigen. Despite this only a few adjuvants have been licensed to human use. over the past decade nanoscale size(<1000nm) materials such as virus-like particles, liposomes, ISCOMs, polymeric, and non-degradable nanospheres have emerged as potential delivery vehicles for vaccine antigens that not only stabilize vaccine antigens but also elicit stronger immune responses. Nanoparticle based vaccines are advantageous concerning the route of delivery. Unlike traditional approached they can be administered through nasal and mucosal routes. However, there are tough challenges to tackle regarding *in vivo* behavior of nanoparticles, antigen release and biodegradability issues before they can be put to human or animal use.

References

1. Xingzhun. Zhonggou yufang yixue sixiang shi (The history of medical thought on prevention in China). Shanghai. 1953, 106-110.
2. Wilson-Welder JH, Torres MP, Kipper MJ, Mallapragada SK, Wannemuehler MJ, Narasimhan B. Vaccine adjuvants: current challenges and future approaches. J Pharm. Sci. 2009; 98:1278-1316.
3. Perrie Y, Mohammed AR, Kirby DJ, Mcneil SE, Bramwell VW. Vaccine adjuvant systems: enhancing the efficacy of sub-unit protein antigens. Int. J Pharm. 2008; 364:272-280.
4. Bolhassani A, Safaiyan S, Rafati S. Improvement of different vaccine delivery systems for cancer therapy. Molecular Cancer. 2011; 10:3-22.
5. Bachmann MF, Jennings GT. Vaccine delivery: a matter of size, geometry, kinetics and molecular patterns. Nat. Rev. Immunol. 2010; 10:787-796.
6. Storni T, Kündig TM, Senti G, Johansen PL. Immunity in response to particulate antigen-delivery systems. Adv. Drug Deliv. Rev. 2005; 57:333-355.
7. Hamdy S, Haddadi A, Hung RW, Lavasanifar A. Targeting dendritic cells with nano-particulate PLGA cancer vaccine formulations. Advanced Drug Delivery Reviews. 2011; 63:943-955
8. Bevan MJ. Understand memory, design better vaccines. Nat. Immunol. 2011; 12:463-465.
9. Beverley PC. Immunology of vaccination. Br Med. Bull. 2002; 62:15-28.
10. Metcalfe SM, Fahmy TM. Targeted nanotherapy for induction of therapeutic immune responses. Trends Mol. Med. 2012; 18:72-80.
11. Mamo T, Poland GA. Nanovaccinology: the next generation of vaccines meets 21st century materials science and engineering. Vaccine. 2012; 30:6609-6611.
12. Moon JJ, Huang B, Irvine DJ. Engineering Nano- and Microparticles to Tune Immunity. Adv. Mater. 2012; 24:3724-3746.
13. Zolnik BS, Gonzalez-Fernandez A, Sadrieh N, Dobrovolskaia MA. Endocrinology. 2010; 151:458-465.
14. Yoo JW, Irvine DJ, Discher DE, Mitragotri S. Bio-inspired, bioengineered and biomimetic drug delivery carriers. Nat. Rev. Drug Discov. 2011; 10:521-535.
15. Burgess R. Stud. Health Technol. Inform. 2009; 149:257-283.
16. Anthony EG, Richard T, Diane Williamson. Vaccine delivery using nanoparticles Front. Cell. Infect. Microbiol, 2013, 13.
17. Zeltins A. Construction and characterization of virus like particles: a review. Mol. Biotechnol. 2012; 53:92-107.
18. Patel KG, Swartz JR. Surface functionalization of virus-like particles by direct conjugation using azide-alkyne click chemistry. Bioconjug. Chem. 2011; 22:376-387 10.1021/bc100367u.
19. McAleer WJ, Buynak EB, Maigetter RZ, Wampler DE, Miller WJ, Hilleman MR. Human hepatitis-B vaccine from recombinant yeast. Nature. 1984; 307:178-180.
20. Agnandji ST, Lell B, Soulanoudjingar SS, Fernandes JF, Abossolo BP, Conzelmann C *et al*. First results of phase 3 trial of RTS, S/AS01 malaria vaccine in African children. N Engl. J Med. 2011; 365:1863-1875.

21. Kanchan V, Panda AK. Interactions of antigen-loaded polylactide particles with macrophages and their correlation with the immune response. Biomaterials. 2007; 28:5344-5357.
22. Steers NJ, Peachman KK, McClain S, Alving CR, Rao M. Liposome-encapsulated HIV-1 Gag p24 containing lipid A induces effector CD4+ T-cells, memory CD8+ T-cells, and pro-inflammatory cytokines. Vaccine. 2009; 27:6939-6949.
23. Guy B. The perfect mix: recent progress in adjuvant research. Nat. Rev. Microbiol. 2007; 5:505-517.
24. Heurtault B, Frisch B, Pons F. Liposomes as delivery systems for nasal vaccination: strategies and outcomes. Expert Opin. Drug Deliv. 2010; 7:829-844.
25. Zhu J, Yan F, Guo Z, Marchant RE. Surface modification of liposomes by saccharides: vesicle size and stability of lactosyl liposomes studied by photon cor relation spectroscopy. J Colloid Interface Sci. 2005; 289:542-550.
26. Zhao W, Wu W, Xu X. Oral vaccination with liposome encapsulated recombinant fusion peptide of urease B epitope and cholera toxin B subunit affords prophylactic and therapeutic effects against *H. pylori* infection in BALB/c mice. Vaccine. 2007; 25:7664-7673.
27. Thomas C, Rawat A, Hope-Weeks L, Ahsan F. Aerosolized PLGA nanoparticles enhance humoral, mucosal and cytokine responses to hepatitis B vaccine. Molecular Pharmaceutics. 2011; 8:405-415.
28. Manish M, Rahi A, Kaur M, Bhatnagar R, Singh S. A single-dose PLGA encapsulated protective antigen domain 4 nanoformulation protects mice against Bacillus anthracis spore challenge. PLoS ONE. 2013; 8:e61885-90.
29. Lu JM, Wang X, Marin-Muller C, Wang H, Lin PH, Yao Q *et al*. Current advances in research and clinical applications of PLGA based nanotechnol ogy. Expert Rev. Mol. Diagn. 2009; 9:325-341.
30. Harikrishnan R, Balasundaram C, Heo MS. Polyd, l-lactide-co-glycolicacid (PLGA)- encapsulated vaccine on immune system in *Epinephelusbruneus* against *Uronemamarinum*. Exp. Parasitol. 2012; 131:325-332.
31. Gordon D, Kelley P, Heinzel S, Cooper P, Petrovsky N. Immunogenicity and safety of AdvaxTM, a novel polysaccharide adjuvant based on delta inulin, when formulated with hepatitis B surface antigen: A randomized controlled Phase 1 study. Vaccine. 2014; 32(48):6469-6477.
32. Debache K, Kropf C, Schutz CA, Harwood LJ, Kauper P, Monney T *et al*. Vaccination of mice with chitosan nanogel-associated recombinant NcPDI against challenge infection with Neospora caninum tachyzoites. Parasite Immunology. 2011; 33:81-94.
33. Lee PW, Hsu SH, Tsai JS, Chen FR, Huang PJ, Ke CJ *et al*. Multifunctional core-shell polymeric nanoparticles for transdermal DNA deliv- ery and epidermal Langerhans cells tracking. Biomaterials. 2010; 31:2425-2434.
34. Xu L, Liu Y, Chen Z, Li W, Liu Y, Wang L *et al*. Surface-engineered gold nanorods: promising DNA vaccine adjuvant for HIV-1 treatment. Nano Letters. 2012; 12:2003-12.
35. Wang T, Zou M, Jiang H, Ji Z, Gao P, Cheng G. Synthesis of a novel kind of carbon nanoparticles with large mesopores and macropores and its application as an oral vaccine adjuvant. Eur. J Pharm. Sci. 2011; 44:653-659.
36. Ow H, Larson DR, Srivastava M, Baird BA, Webb WW, Wiesner U. Bright and stable core-shell fluorescent silica nanoparticles. Nano Letters. 2005; 5:113-7.
37. Benezra M, Penate-Medina O, Zanzonico PB, Schaer D, Ow H, Burns A *et al*. Multimodal silica nanoparticles are effective cancer-targeted probes in a model of human melanoma. J Clinical Investigation. 2011; 121:2768-80.
38. Niu Y, Popat A, Yu M, Karmakar S, Gu W, Yu C. Recent advances in the rational design of silica-based nanoparticles for gene therapy. Therapeutic Delivery. 2012; 3:1217-37.
39. Barr IG, Sjölander A, Cox JC. ISCOMs and other saponin based adjuvants. Adv. Drug Deliv. Rev. 1998; 32: 247-271.
40. Coulter A, Harris R, Davis R, Drane D, Cox J, Ryan D *et al*. Intranasal vaccination with ISCOMATRIX® adjuvanted influenza vaccine. Vaccine. 2003; 21:946-9.
41. Mohamedi S, Brewer J, Alexander J, Heath A, Jennings R. Antibody responses, cytokine levels and protection of mice immunized with HSV-2 antigens formulated into NISV or ISCOM delivery systems. Vaccine. 2000; 18:2083-94.
42. Agrawal L, Haq W, Hanson CV, Rao DN. Generating neutralizing antibodies, Th1 response and MHC non restricted immunogenicity of HIV-I env and gag peptides in liposomes and ISCOMs with in-built adjuvanticity. J Immune Based Therapies and Vaccines. 2003; 1:5-26.
43. Kersten GF, Spiekstra A, Beuvery EC, Crommelin DJ. On the structure of immune-stimulating saponin-lipid complexes (iscoms). Biochim. Biophys. Acta. 1991; 1062:165-171.
44. Peek LJ, Middaugh CR, Berkland C. Nanotechnology in vaccine delivery. Advanced Drug Delivery Reviews. 2008; 60:915-28.
45. Kumar S, Jones TR, Oakley MS, Zheng H, Kuppusamy SP, Taye A *et al*. CpG oligodeoxynucleotide and Montanide ISA 51 adjuvant combination enhanced the protective efficacy of a subunit malaria vaccine. Infection and Immunity. 2004; 72:949-57.
46. Kanekiyo M, Wei CJ, Yassine HM, McTamney PM, Boyington JC, Whittle JR *et al*. Self-assembling influenza nanoparticle vaccines elicits broadly neutralizing H1N1 antibodies. Nature. 2013; 499:102-6.
47. Champion CI, Kickhoefer VA, Liu G, Moniz RJ, Freed AS, Bergmann LL *et al*. A vault nanoparticle vaccine induces protective mucosal immunity. PLoS ONE. 2009; 4:e5409.
48. Unal CM, Schaar V, Riesbeck K. Bacterial outer membrane vesicles in disease and preventive medicine. Semin Immunopathol. 2011; 33:395-408.
49. Kim JY, Doody AM, Chen DJ, Cremona GH, Shuler ML, Putnam D *et al*. Engineered bacterial outer membrane vesicles with enhanced functionality. J Mol. Biol. 2008; 380:51-66.
50. Mody KT, Popat A, Mahony D, Cavallaro AS, Yu C, Mitter N. Mesoporous silica nanoparticles as antigen carriers and adjuvants for vaccine delivery. Nanoscale. 2013; 5:5167-79.
51. Oyewumi MO, Kumar A, Cui Z. Nano-microparticles as immune adjuvants: correlating particle sizes and the resultant immune responses. Expert Review of Vaccines. 2010; 9:1095-107.
52. Wendorf J, Singh M, Chesko J, Kazzaz J, Soewanan E, Ugozzoli M *et al*. A practical approach to the use of nanoparticles for vaccine delivery. J Pharmaceutical Sciences. 2006; 95:2738-50.

53. Slutter B, Soema PC, Ding Z, Verheul R, Hennink W, Jiskoot W. Conjugation of ovalbumin to trimethyl chitosan improves immunogenicity of the antigen. J Controlled Release. 2010; 143:207-14.
54. Xiang SD, Scholzen A, Minigo G, David C, Apostolopoulos V, Mottram PL *et al*. Pathogen recognition and development of particulate vaccines: does size matter. Methods. 2006; 40:1-9.
55. Joshi VB, Geary SM, Salem AK. Biodegradable particles as vaccine delivery systems: size matters. AAPS J. 2013; 15:85-94;
56. Foged C, Brodin B, Frokjaer S, Sundblad A. Particle size and surface charge affect particle uptake by human dendritic cells in an *in vitro* model. Int. J Pharmaceutics. 2005; 298:315-22.
57. Nakanishi T, Kunisawa J, Hayashi A, Tsutsumi Y, Kubo K, Nakagawa S *et al*. Positively charged liposome functions as an efficient immunoadjuvant in inducing cell-mediated immune response to soluble proteins. J Controlled Release. 1999; 61:233-40.
58. Champion JA, Mitragotri S. Role of target geometry in phagocytosis. Proceedings of the National Academy of Sciences of the United States of America. 2006; 103:4930-4.
59. Champion JA, Mitragotri S. Shape induced inhibition of phagocytosis of polymer particles. Pharmaceutical Research. 2009; 26:244-9.
60. Niikura K, Matsunaga T, Suzuki T, Kobayashi S, Yamaguchi H, Orba Y *et al*. Gold nanoparticles as a vaccine platform: influence of size and shape on immunological responses *in vitro* and *in vivo*. ACS Nano. 2013; 7:3926-38.
61. Hillaireau H, Couvreur P. Nanocarriers' entry into the cell: relevance to drug delivery. Cellular and Molecular Life Sciences. 2009; 66:2873-96.
62. Banchereau J, Steinman RM. Dendritic cells and the control of immunity. Nature. 1998; 392:245-252.
63. Demento SL, Bonafe´ N, Cui W, Kaech SM, Caplan MJ, Fikrig E *et al*. TLR9-targeted biodegradable nanoparticles as immunization vectors protect against West Nile encephalitis. J Immunol. 2010; 185:2989-2997.
64. Aggarwal P, Hall JB, McLeland CB, Dobrovolskaia MA, McNeil SE. Nanoparticle interaction with plasma proteins as it relates to particle biodistribution, biocompatibility and therapeutic efficacy. Advanced Drug Delivery Reviews. 2009; 61:428-37.
65. Reddy ST, van der Vlies AJ, Simeoni E, Angeli V, Randolph GJ, O'Neill CP *et al*. Exploiting lymphatic transport and complement activation in nanoparticle vaccines. Nature Biotechnology. 2007; 25:1159-64.
66. Swartz MA. The physiology of the lymphatic system. Advanced Drug Delivery Reviews. 2001; 50:3-20.
67. Manolova V, Flace A, Bauer M, Schwarz K, Saudan P, Bachmann MF. Nanoparticles target distinct dendritic cell populations according to their size. European J Immunology. 2008; 38:1404-13.
68. Longmire M, Choyke PL, Kobayashi H. Clearance properties of nano-sized particles and molecules as imaging agents: considerations and caveats. Nanomedicine. 2008; 3:703-17.
69. Ohlson M, Sörensson J, Haraldsson B. A gel-membrane model of glomerular charge and size selectivity in series. American J Physiology Renal Physiology. 2001; 280:F396-405.
70. Arora S, Rajwade JM, Paknikar KM. Nanotoxicology and *in vitro* studies: the need of the hour. Toxicology and Applied Pharmacology. 2012; 258:151-65.
71. Huang X, Li L, Liu T, Hao N, Liu H, Chen D *et al*. The shape effect of mesoporous silica nanoparticles on biodistribution, clearance, and biocompatibility *in vivo*. ACS Nano. 2011; 5:5390-9.
72. Harrison RL, Jarvis DL. Protein N-glycosylation in the baculovirus insect cell expression system and engineering of insect cells to produce mammalianized recombinant glycoproteins. Adv. Virus Res. 2006; 68:159-191.
73. Soppimath KS, Aminabhavi TM, Kulkarni AR, Rudzinski WE. Biodegradable polymeric nanoparticles as drug delivery devices. J Control. Release. 2001; 70:1-20.
74. NIOSH. Approaches to safe nanotechnology, in Department of Health and Human Services, National Institute for Occupational Safety and Health, ed Centers for disease control and Prevention. 2006 Available online at: http://www. cdc.gov/niosh/docs/2009-125/pdfs/2009-2125.
75. Oberdorster G, Ferin J, Lehnert BE. Correlation between particle size, *in vivo* particle persistence and lung injury. Environ. Health Perspect. 1994; 102(5):173-179.
76. Tsoli M, Kuhn H, Brandau W, Esche H, Schmid G. Cellular uptake and toxicity of Au55 clusters. Small. 2005; 1:841-844.
77. Fitzpatrick JA, Andreko SK, Ernst LA, Waggoner AS, Ballou B, Bruchez MP. Longterm persistence and spectral blueshifting of quantum dots *in vivo*. Nano Lett. 2009; 9:2736-2741.
78. Stephen A, Kaba Clara Brando, Qin Guo, Christian Mittelholzer, Senthilkumar Raman, David Tropel *et al*. A non adjuvanted polypeptide nanoparticle vaccine confers long-lasting protection against rodent Malaria. J Immnol. 2009; doi/10.4049.
79. James J Moon, Heikyung Suh, Mark E Polhemus, Christian F Ockenhouse, Anjali Yadava, Darrell J Irvine. Antigen displaying lipid enveloped PLGA nanoparticles as delivery agents for a *Plasmodium vivax* malaria vaccine. 2012; 7:2e 31472.
80. Saljoughian N, Zahedifard F, Doroud D, Doustdari F, Vasei M, Papadopoulou B *et al*. Cationic solid–lipid nanoparticles are as efficient as electroporation in DNA vaccination against visceral leishmaniasis in mice. Parasite Immunology. 2013; 35:397-408.
81. Carolina R Oliveira, Cıntia MF Rezende, Marina R Silva, Olga M Borges, Ana P Pego, Alfredo M Goes. Oral Vaccination Based on DNA-Chitosan Nanoparticles against *Schistosoma mansoni* Infection. The scientific world J 2012; Article ID 938457. doi:10.1100/2012/938457.
82. Jang SI, Lillehoj HS, Lee SH, Lee KW, Park MS, Bauchan GR *et al*. Immunoenhancing effects of Montanide ISA oil-based adjuvants on recombinant coccidian antigen vaccination against *Eimeria acervulina* infection. Vet. Parasitol. 2010; 17