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Review Article

Vaccine Delivery Using Nanoparticles: A Critical Look at ISCOMs 4 Decades but 2 and Still Counting

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ABSTRACT

The immunostimulatory complexes (ISCOMs), a cage-like nanoparticle, was discovered about 38 years ago. It is a competent antigen delivery system with high level of versatility in terms of its modes of action (promotion of both B- and T-lymphocytes activities) and routes of administration (mucosal and parenteral routes). ISCOMs is a nanoparticle with an average size of between 30-60 nm and composed of saponins, cholesterol, phospholipids and antigen. In this review we discussed the potentials of ISCOMs as a good candidate for vaccine adjuvant in veterinary animals and humans. We also looked at the challenges facing the development of licensed human vaccines containing ISCOMs as adjuvant and the future of the wonderful molecule.

Keywords: Adjuvant, Iscoms, Iscomatrix, Nanoparticles, Vaccines.

Introduction

Vaccine development has had an appreciable impact on global health as the world had recorded great achievements in the area of reduction/total elimination of some virulent disease(s). To this effect, the importance of vaccine development cannot be over emphasized especially in this era of emerging viral infectious diseases.

In the last decade, the global community has been constantly challenged with one viral outbreak or the other that have led to loss of millions of lives. It is therefore imperative that scientist should exploit the discoveries in the area of molecular biology, bioinformatics, biotechnology and extended understanding of the immune system in the development of sophisticated vaccine candidates for emerging infectious diseases (EIDS) as well as complex diseases. Unlike the old generation vaccines which are either whole cell inactivated or live attenuated, the new generation vaccines are usually made up of antigen fragments prepared from either the disease-causing organisms themselves or via biotechnological procedures. It has been established, that vaccines formulated using antigen fragments of the disease causing organisms are usually not able to stimulate strong immune response when compared to vaccines formulated with whole organisms (attenuated or killed).[1] To take care of these shortcomings, and also achieve the aim of vaccination which is principally the stimulation of B and T-lymphocytes to generate immune response against the antigen administered, an adjuvant is usually added. The credit of the discovery of immunestimulatory complexes (ISCOMs) goes to Morein and his colleagues in 1984. They took advantage of the small size of viral particles and arrangements of their surface proteins in addition to the inert immunostimulatory potentials of saponins to design this vaccine delivery system.[2] These properties can be linked with the robust immune responses experienced from ISCOMs in most animal studies. ISCOMs as a vaccine adjuvants are known to generate stronger immune response when compared with other adjuvants.[3,4] ISCOMs are nanoparticles of average size of 40nm composed of phospholipids, cholesterol, saponin mixture, Quil A and an antigen.[5,6] ISCOMs usually occur in two variant forms, first is the one described above and the second form is essent ially the same structure with the first but without the antigenic protein and it is usually referred to as ISCOM matrix.

Peparation of ISCOMs

ISCOMs are colloidal particles composed of various ratios of cholesterol, phospolipids, saponins mixture Quil A and antigen. There are various methods that can be used to prepare ISCOMs and the methods are as explained below:

Methods of preparation of ISCOM

Dialysis method

This is the most common method adopted by scientists in the preparation of ISCOMs. It involves equipments such as Rotavapor, magnetic stirrer, dialysis tubing etc. Various detergents (sodium cholate, n-octylglucopyranoside, polyoxyethylene ethers or phenyl ethers, Triton X-100 (octylphenolether of polyethylene oxide), acylpolyoxyethylene esters, acyl polyoxyethylenesorbitan esters (Tween series), the span series, Ionic detergents such as the gallic acid detergents (bile salts).)[7] can be used in the preparation of ISCOMs. According to Demana *et al.*,[8] a typical dialysis method involve dissolution of various amounts of phospholipids e.g phosphotidyl ethanolamine (PE) and Cholesterol in Chloroform and evaporated at 45^{0}C for 1hr with the aid of rotavapor. The dried lipid film will be mixed with different concentrations of Quil A in Tris buffer. Octylglycoside for example acting as detergent will be used to solubilize the water-soluble lipids. Protein or modified proteins at given concentrations will be added to the mixture. With the aid of a magnetic stirrer, the mixture in micellar form will be stirred for 5 hrs at room temperature. The samples will then be placed in dialysis tubing and dialyzed against several changes Tris buffer at 4^{o}C for 3 days to remove the detergent (octylglucoside).

Reverse phase evaporation technique

This is another method of ISCOMs preparation according to.[9] In this technique an organic phase will be prepared by dissolving phosphotidylcholine (PC) and cholesterol (chol) in diethyl ether. An aqueous phase will be prepared by dissolving saponin (Quil

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A) and antigen in a phosphate buffer solution (PBS, pH7.4). The organic phase will then be added drop wise to the aqueous phase under ultrasonication at amplitude 20 for 1: 30 minutes this is preceded with vortexing of the solution for 20 runs to eliminate the residual organic solvent.

Lipid film hydration method
This is a simple procedure when compared to the complex nature of the dialysis or centrifugation method. It is simply the addition of aqeous solution of Quil A to cholesterol/phospholipid film already formed. The procedure involves the dissolution of various amounts of cholesterol and phospholipids in chloroform and evaporated to dryness with the aid of a Rotavapor. Aqeous solution of Quil A will be added to the dried lipid film with gentle mixing for about 20 mins at room temeperature. The solution formed may be analysed immediately or subjected to centrifugation through a 10 – 50% sucrose gradient to fractionate it before analyzing.[10]

Ethanol injection method
This method is similar to the already described method of preparation of unilamellar liposomes by the ethanol injection method. Firstly mixture of cholesterol and phospholipids are dissolved in ethanol to form an ethanolic solution. The resulting solution are injected into aqeous solution of Quil A or a mixture of Quil A and antigen followed by stirring.This method results in the formation of ISCOMs within 24 -48 hrs. The method is simple, rapid, efficient, and offers the possibilities for large scale commercial production.[11]

Ether injection method
In this method, phosphatidyl choline and cholesterol will be dissolved in ether, which will be injected into an aqueous solution of Quil A, maintained at 55^0C. The use of ether has the advantage that higher quantities of lipids can be dissolved and that removal of the organic solvent is achieved by gentle heating.

Advantages of ISCOMs as Vaccine adjuvants
The importance of ISCOMs as vaccine adjuvants can be linked to the following advantages:

1. They generate strong and long lasting immunological responses.[12]
2. ISCOMs produce strong immunological response at reduced antigen dose hence reduction in production cost of vaccine manufacture.[12]
3. ISCOM-borne antigens has been shown to possess great access to the cytoplasmic matrix of antigen presenting cells which will ultimately lead to robust immunological response/stimulation.[14,15]
4. They have been shown to be versatile with respect to routes of administration such as mucosal and parenteral routes.[16]
5. ISCOMs have been extensively used in veterinary medicine with positive results e.g horses, ruminants, chickens, dogs, cats etc.[17]
6. They may be used as adjuvants in neonatal vaccines as they have shown the ability to produce immune response without interference from passively derived maternal antibodies.[18]

Disadvantages of ISCOMs as vaccine adjuvant
1. 1.The two most common methods for the preparation of ISCOMs (are centrifugation and dialysis) are very elaborate and complex.
2. It is also an expensive procedure.
3. 3.The issue of hemolytic toxicity associated with saponin which is the basic component of ISCOMs has limited its use in humans and some other animal species.

Characterization of Iscoms
It is essential to characterize the composition and structure of formulations of ISCOMs to avoid intra or inter batch variations. These variations could arise from contamination, accumulation of toxic components or incomplete particle formation. In order to maintain uniformity, methods such as transmission electron microscopy, photon correlation spectroscopy and density gradient centrifugation are utilized to characterize the shape and size of particles in ISCOMs formulation.[19] Using techniques such as Enzyme-linked immunosorbent assay, dot-blots, density gradient centrifugation, western blotting, sodium dodecyl sulphate polyacrylamide gel electrophoresis, fluorescence spectroscopy and Lowry and Bradford assays, the amount of protein or antigen incorporated in the formulation can be determined.[20,21] It js also important to determine the amount of other substances wspecially if they exhibit toxicities at high doses. A typical example is the hemolytic activity produced by Quil A, a component of ISCOMs at high dose. The amount of Quil A can be measured using reversed phase high-performance liquid chromatography or rocket electrophoresis assay.[19] Phospholipids are measured by phosphorus assay while cholesterol can be quantified by gas chromatography.[19]

Morphology of ISCOMs
The morphology of ISCOMs can be determined using Transmission Electron Microscopy (TEM).[8] Carbon-coated copper grids are glow-discharged (Edwards E 306 A vacuum coater, England) and 10µl of sample adsorbed on to these grids. The samples are negatively stained using 10µl of filtered 2% phosphotungstic acid, PH 5.2 as a contrast agent. Samples are investigated using a Philips CM 100 transmission electron microscope at an acceleration voltage of 100kv and are typically viewed at a magnification of 135,000 ×. The size of the colloidal structure is determined using Analysis software (soft imaging systems, Reutlingen, Germany). The observed particles are measured, prevalence of different colloidal structures estimated and expressed as percentage of the colloidal particles present in the sample.

Determination of size of ISCOMs
Photon correlation spectroscopy is an analytical tool used to measure the size of macromolecules and colloids in solutions.[22] It employs the variation in intensity of scattered light on the microsecond time scale. This variation results from the interference of light scattered by individual particles under the influence of Brownian motion and is quantified by compilation of an auto correlation function. This function is fitted to an exponential, or some combination or mordification thereof with the corresponding decay constant(s) being related to the diffusion coefficients. Particle size can be calculated from this coefficient using standard assumptions of spherical size, low concentration, and known viscosity of the suspending medium. Rapid analysis, lack of required calibration and sensitivity to submicron sized particles are some of the advantages of this method. The molecular weight of each nanoparticle can be estimated through the utilization of spectral decomposition capacity of dynamic light scattering.[23]

Determination of the amount of incorporated protein
Fluorescence spectroscopy can be exploited in the determination of the amount of protein/antigen incorporated into ISCOMs and other colloidal particles.[20] This method involves the disruption of the colloidal structures by addition of about 50µl of the dispersion to 750µl Tris buffer (PH 6.6) containing 5% Triton X-100. Fluorescence of the resulting solution is measured (Shimadzu FR 540, ex.495nm, em,518nm) and entrapment efficiency of protein into various colloidal particles are estimated based on standard curves constructed for each protein. The amount of protein or mordified protein incorporated into the colloidal particles, is then calculated and expressed as a percentage of the original amount of protein or mordified protein used per µmol of lipid.

Applications of ISCOMs
ISCOMs and vaccine delivery in veterinary medicine

The use of ISCOMs as adjuvants in laboratory animals have recorded successes according to several studies. The last two decades have witnessed studies on immunogenicity and protection from ISCOMs-containing antigens and antigenic derivatives from different variety of pathogens, such as viruses, bacteria, protozoas etc. Although the experiments on the use of ISCOMs as adjuvants were originally carried out using mice or rats in the laboratories, It has also shown the ability to induce strong immunological responses in other species.[18,24,25]

ISCOMs-based vaccines in Dogs

Programs supporting regular vaccination in dogs have contributed both to the health of dogs and to the public health. In areas where routine rabies vaccination of dogs is practiced, rabies in humans has been shown to be drastically reduced if not eliminated. Rabies virus which belong to the Rhabdoviridae family has been associated with serious disease in warm- blooded animals. Though not made commercially, rabies virus vaccines have incorporated ISCOMs with positive outcomes according to the study.[26] Cystic echinococcosis is a global public health problem and the disease is transmitted to man from dogs. Vaccines have been shown as a sure way of preventing the transmission. A study of the ISCOMs vaccine formulation for the prevention of echinococcosis produced good protection against the disease.[27]

Canine Distemper Virus (CDV) is another virus which causes disease in dogs that presents with similarity to measles in man. The CDv which is inhaled by dogs are carried by the macrophages to lymphnodes where replication begins. In a matter of 2-5 days it has affected other lymphoid organs through rapid spread in the lymphatic tissues.[28] By day 6-9, the virus spreads to the blood (viremia). Having spread to the blood, soon it gets across other parts of the body such as epithelium of the gastrointestinal, central nervous system, urogenital, and respiratory systems. The animal then comes down with the full infection presenting symptoms such as mild eye inflammation, loss of appetite and fever that may only last a few days (http://acadogs.com/canine_Distemper). These symptoms become more pronounced as the disease progresses. Canine distemper virus vaccine formulated using ISCOMs as adjuvants protected dogs against high blood virus levels and other signs of infections.[29] This is very interesting considering the pathogenesis of CDV as described above. Another area of great interest is the ability of ISCOMs to produce high level of protective antibody in young puppies less than 8weeks of age area commercial vaccines have failed. This is due to immature immune system and the blocking effect of maternal antibodies.[17]

ISCOMs based vaccines in cattle

Experimentally, ISCOMs have been extensively studied for use in the production of various vaccines in cattle with high percentage of successes recorded. A few of such ISCOMs are discussed below:

Rinderpestvirus (RVP) a member of genius *morbillivirus* in the family *paramyxoviridae* is associated with severe diseases often with high morbidity and mortality in cattle and other large ruminants. An experimental rinderpest vaccine containing immune stimulating complex and the RVP haemagglutinin(H) protein, was studied for its potential to cause an immunological protection to the immunized cattle, and it was found to be effective eliciting the generation of high level of anti-RVP antibodies.[30]

In separate experiments in Canada and Hungary, Bovine herpes virus ISCOMS were shown to fully protect the experimental animals.[31] This virus is associated with diseases in cattle such as balanoposthitis, abortion, enteritis, rhinotracheitis, vaginitis and conjunctivitis. Bovine Viral Diarrhea Virus (BVDV) is a viral infection that affect cattle of all ages. It also affect fetus leading to possible conditions such as teratogenicity, persistent infection in the neonatal calf, abortions or stillbirths. An experimental subunit ISCOMs vaccine against BVDV capable of inducing high serum neutralizing antibodies was developed.[32] Bovine respiratory syncytial virus has been associated with severe respiratory tract infection both in Man and Cows. An experimental BRSV ISCOMs vaccine induced rapid humoral immune responses and strong clinical and virological protection against experimental BRSV challenge.[33]

Contagious Bovine Pleuropneumonia (CBPP also known as lung plague) is a bacterial disease of cattle caused by the bacterium *Mycoplasma mycoides*. It is contagious and causes pneumonia and inflammation of the lung membrane. Experimentally, CBPP ISCOMs was shown to induce strong primary and long lasting secondary antibody responses although the result in the field was not encouraging.[34]

ISCOMs based vaccines for horses

The influenza virus that attack horses belongs to the family known as *orthomyxoviridae*. It is highly contagious diseases that primarily affect the respiratory tract of horses with resultant high morbidity and sometimes mortality. The predominant subtypes of this virus seen in horses include H7N7 and H3N8.[35] The H7N7 EIV may have gone into extinction as it has not been seen in samples tested for over 20 years.[36,37] The H3N8 EIV is the subtype associated with global influenza outbreak in horses,[38] even when the animals are vaccinated. The vaccine used are usually whole attenuated virus or its fragment which are known to generate antibodies against the viral pathogen.[39] However the shortcoming of this immune response is that it is short lived and highly specific. The recirculation may be linked to the short-lived nature of the conventional vaccine and also its high specificity which do not take care of variants strains of the virus. Equine influenza vaccines containing ISCOMs as adjuvants have been extensively experimented on for several years. The results generated from the experiments showed the ability of the vaccines to induce high and long-lasting humoral and cellular immunity when compared with the conventional killed whole Influenza virus vaccine.[40,41] Based on elaborate and extensive study of ISCOM vaccines containing EIV antigens with a lot of success stories and efficacy, the first licensed and commercially available ISCOMs vaccine was used in horses and it contains the envelope protein haemaglutinin (HA) and neuraminidase (NA) from influenza virus.[42] This vaccine was produced by ISCOTEC AB and Mallinckrodt (UK) in 1989 and over 1 million doses of the vaccine have been sold in Sweden with beautiful outcomes on the animals administered.[43]

Other animals

ISCOMs-based vaccines has shown some successes in cat and chicken. Experimental ISCOMs delivery containing feline immunodeficiency virus (FIV) and *Eimeria tenella* antigen have been successfully formulated for use incat and chicken respectively. The formulation was shown to stimulate host immune response while demonstrating low toxicity in the challenged animals.[44,45]

ISCOMs and vaccine delivery in non-human primates

The non-human primates play active role in biomedical research. This is because they share about 98% of human genes and can be used to extrapolate the causes, progression, prevention and treatment of a wide variety of diseases of man. The anatomy, body physiology and character of these animals are similar to that of humans hence provide an important link between basic laboratory studies and clinical use (California Biomedical Research Association, Fact sheet: primates in Biomedical Research: www.cabiomed.org). Measles virus ISCOMs have been extensively studied both as subunit and inactivated vaccines in no human primates.[46] This measles ISCOMs based vaccine was associated with strong IgG, IgM production and cytotoxic T-lymphocyte proliferation in Macaque model.[47] Influenza virus ISCOMs has also been shown to be safe after administration in monkeys without signs of toxicity. ISCOMs based vaccine containing influenza virus antigen demonstrated high level of antibody-mediated protective immunity and antigen- specific proliferative T-cell responses in monkeys after immunization.[48] Semian immunodeficiency virus (SIV) share close resemblance to human immunodeficiency virus (HIV) in terms of their genomic organization hence can induce disease in monkeys similar to human AIDS. Therefore, SIV infection of these primates have been adopted

by scientists as model for researches on HIV vaccines.[49] Immunizations of macaques with HIV envelope glycoproteins and SIV ISCOMs have shown their ability to induce protective immunity. This protective immunity may be linked to the ability of the various ISCOMs based vaccine to induce production of antibodies, T-cell and CTL proliferation.[50,51,52] There are other infectious diseases where ISCOMs have demonstrated high level of avidity/protection experimentally in non human primates and these include Hepatitis C virus(HCV),[53]Epstein-Barr[54] (EBV), falciparum malaria,[55]and Japanese encephalitis virus.[56]

ISCOMs and vaccine delivery in humans

The development of licensed vaccines meant for human use is more complex when compared with vaccines for veterinary or other animals. This is because special attention is paid to the issue of potency versus safety and detailed unambiguous defined manufacturing procedures that is reproducible. As a result of the aforementioned, Aluminium-based adjuvant which was discovered since 1926 has continued to monopolize human vaccines.[57] However the last decade has witnessed introduction of newer vaccine adjuvants for human use. They include MF59 (composed of squalene droplets stabilized with surfactants Tween 80 and Span 85),[58] squalene- based adjuvant AS03,[59] AS04 (monophosphoryl lipid A (MPL) + alum),[60] AF03 (squalene- based emulsion adjuvant), virosomes and heat-labile enterotoxin (LT).[61] With respect to this review, there is no licensed ISCOMs-based vaccine for human use. However about eight phase I or II randomized placebo or Ag controlled studies evaluating vaccines using *Iscomatrix* adjuvant have been completed although six studies are ongoing in either prophylactic or therapeutic vaccine programs for infectious diseases or cancer. Of the completed studies, six studies have evaluated Iscomatrix vaccines via the I.M route, one study assessed an intranasal formulation and another study evaluated the effects of Iscomatrix on pulsed blood dendritic cells *ex vivo*. The studies showed a successful outcome as the vaccines were efficacious, safe with minimal side effects in the human patients.[62] Two vaccines containing ISCOMs PANFLUVAC (H5N1) vaccine and NY-ESO-1 ISCOMATRIX (Melanoma vaccine) are in phase I and phase II clinical trials respectively.[63]

Modes of action of ISCOMS

Antigen targeting and presentation

Several laboratory studies have confirmed the ability of antigen presenting cells (dendritic cells, macrophages) to bind ISCOMs containing antigen and effectively present them to T-cells.[64,65] The popularity of ISCOMs as vaccine adjuvants have been linked to its ability to positively manipulate immune cells. There is a postulation that ISCOMs can deliver antigen directly to the cytosol of antigen presenting cells where the antigens are degraded and loaded inside the MHC class 1 in the endoplasmic reticulum for cytotoxic T-lymphocyte activation.[66,67,68] This ability of ISCOMs to pass antigens through the membranes is linked to its hydrophobic nature in addition to the presence of saponins that has affinity for membrane bound cholesterol.

Cytokine production

The term "cytokine" is derived from a combination of two Greek words—"cyto" meaning cell and "kinos" meaning movement. Cytokines are cell signaling molecules that aid cell to cell communication in immune responses and stimulate the movement of cells towards sites of inflammation, infection and trauma (MandalAnanya www.news-medical.net). Based on this definition, one can appreciate that the capacity of adjuvants such as ISCOMs to stimulate cytokine production by Antigen-presenting cells is important for the initiation of the immune response. Studies using various animal models have revealed the ability of ISCOMs to stimulate the production of inflammatory cytokines interleukin-1(IL-1), interleukin-2(IL-2) and tumor necrosis factor (TNF-α).[69,70,71] Another study also show its ability to stimulate the production of interleukin-6(IL-6), interleukin-8(IL-8) and IFN-ɣ.[72]

Proliferation of antibodies and T-cells

ISCOMs modes of action also reflects it versatility. Its effects on antigen targeting and presentation and on cytokine production as discussed above are all associated with the innate component of the immune system. Antibodies and T-cells which are the two components of the acquired immune system are greatly affected by ISCOMs. The ability of ISCOMs to induce high and long lasting antibody secretion and elevation of both helper T-cells and cytotoxic T-cells have been described in some studies.[12,73-75]

Modes of administration of iscoms

The ability of a vaccine candidate to elicit the desired protection expected of it, it should be able to reach and interact with immune-active cells distributed throughout the body. These immune-active cells are distributed both systemically and on mucosal surfaces. Though it is common knowledge that most infections occur through the mucosal surfaces, the parenteral routes of administration of vaccines seem to be more popular than other routes. However, since most infections take place through the mucosal membranes, and the first line of defense often lies in the mucus containing secretory IgA antibodies,[76] vaccine delivery to this site will enhance defense provided by these IgA antibodies.

ISCOMs and mucosal vaccine delivery

The mucosal route is basically oral, intranasal, transdermal routes etc. The mucosal route has an important characteristic of not limiting the response to the site of administration due to a phenomenon known as induction of common mucosal immune system (CMIS).[77] The CMIS stimulation will cause the immune effector cells to relocate to mucosal surfaces where they provide protection against pathogens at these portals of entry. Extensive animal studies spanning from small animal models (mice) to larger animals (dogs, monkeys etc) have shown that ISCOMs based vaccines are highly immunogenic after oral or intranasal administration. The studies on intranasal route were larger and more extensive than the oral routes. Researchers have attested to the immunogenicity of intranasal administered ISCOMs based vaccine.[78-82] Similarly several studies support the fact that ISCOMs based vaccines can be given orally.[69,83,84] Despite the above mentioned *in vivo* animal studies, an *in vitro* study carried out on a human colon epithelial cell line, (Caco-2 cells) by[85] showed that ISCOMs containing influenza virus antigen was taken up via the apical surface and the antigens were processed and transported through the basal membrane.

ISCOMs and parenteral vaccine delivery

The early works on ISCOMs which laid down the foundation of ISCOMs as adjuvants for vaccine delivery were done through the parenteral routes. This gave impetus to scientists to try ISCOMs as a mucosal vaccine delivery system. This can be corroborated by works done by.[39,86,87,48]

Iscoms based vaccines for commercial use

ISCOMs based vaccines have been faced with enormous challenges that have hindered their movement from the laboratory to the clinics both in animals and humans. The two ISCOMs based vaccines translated for commercial uses are the vaccines for influenza virus in horses (Table 1).

Major challenges of ISCOMs based vaccines

Just like conventional drug development, the risks to benefits ratio of adjuvants incorporation into vaccines need to be considered which serve as a guide for its selection. Iscoms based vaccines despite large studies in the laboratory animals have not been able to move from the laboratories to clinics.

Table 1: Commercially available Iscom based vaccines

Trade Name	Company	Technology	Reference
Equip-F	Pfizer Ltd	Inactivated	[39]
EquilisPrequenza	Intervet/Schering	Inactivated	[42]

	plough anumal
	health

This is because of saponins-associated hemolysis and granulomas toxicities.[88] ISCOMs-based vaccines when administered intravenously, intraperitoneal and subcutaneously showed high toxicities in murine models of animal studies at an LD_{50} of 0.67mg/kg.8[9,90] However, it is worthy of note that this toxicity was very minimal in larger terrestrial animals such as but more pronounced in laboratory animals.[1,91] Another bottle neck inhibiting the emergence of ISCOMs vaccine in humans is the issue of regulatory barriers. Elaborate and extensive toxicological studies are carried out not only on the adjuvant but also on the adjuvant-antigen formulation using laboratory animals before entering phase-1 clinical trials.[92] Another tedious regulatory barrier is the population size to be tested for efficacy and safety of the vaccine adjuvant. The number has increased geometrically in recent years to take care of some drug candidates which may have rare but fatal side effects that may not be identified if the population size is not adequate.

Conclusion and future perspectives

The importance of extended research towards the development of a safe, efficacious and versatile vaccine adjuvant especially in this era of subunit vaccines cannot be overemphasized. This is because of the poor immunogenic nature of the new generation vaccine when compared to the conventional vaccine hence the need for a boost (adjuvant). The emergence of ISCOMs in 1984 was greatly welcomed by the vaccine world. This is because of high popularity of aluminium salts as adjuvants in human vaccines despite its inability to induce cellular immunity. It is clear that from some of the research concluded and on-going, that ISCOMs have continuously proven to be versatile with respect to stimulating both natural and acquired immune responses and also effective through multiple routes of administration. However, it is worrisome that a particle with such characteristic has not been licensed for use in human vaccines. We therefore recommend that robust research be carried out on ISCOMs using saponins extracted from other medicinal plants with wild application in alternative medicine. This approach may help address issues of toxicities compatibilities and stability so as to fully move this notable molecule from the laboratories to a potent, safe and efficacious animal and human therapeutic agent.

Conflict of Interest

Authors declare no conflict of interest

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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