Mini Review

Vaccine development and delivery strategies – A glimpse

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Abstract

Vaccines are one of the oldest biological products which are most successful in modulating immune response against various life-threatening infectious diseases. Currently, there are many advancements in the designing and delivery of vaccines. Starting from the use of live attenuated and dead microbes as vaccines, to the stage of mapping antibodies using various software and algorithms for designing desired vaccine, the journey has taken many turns in the last two centuries. Current write up gives a glimpse of various developments in the development of vaccines as well as delivery strategies like use of immunoinformatics, artificial intelligence and efforts made towards the delivery of vaccines using nanoparticles, vectors, hydrogels as adjuancts.

Introduction

Vaccines are the biological products used to provide active immunity to infectious diseases. It is a well-known fact that generally vaccines are made either of killed or attenuated microbes, toxoids, virulent protein subunits, or polysaccharide outer coats along with proteins or toxins. The history of vaccines dates back to 1798 when Edward Jenner gave smallpox exudates to an 8-year-old boy who did not get smallpox after being infected [1]. Since then, research on vaccines underwent technological as well as medical changes, and several vaccines, as well as antitoxins for diseases like diphtheria, tetanus, plague, smallpox, rabies, cholera, were developed by the mid of 20th century. Mass vaccination was introduced by the World Health Organization (WHO) for smallpox and its success prompted many regulatory agencies to make guidance and regulations for the discovery, production, and usage of vaccines.Thereby sales of viruses, serums, toxins, and related products were brought under regulations in the 20thcentury [2]. During that period when vaccines were made available, high-income countries began recommending routine vaccination for children. Currently, in most countries, vaccination is mandatory for children. The widespread success of vaccination proved that vaccines are lifesaving interventions. This paper tries to shed light on few developments in vaccine development and delivery technologies.

Advancements in vaccine technology

Across decades, with occurrence of newer diseases – infectious / non-infectious / allergic there is a constant need for better therapeutic and treatment options. Prevention of the occurrence of the disease is a desirable option. Secondly, with the global population explosion, the preventive therapy calls for large scale production of the therapeutic agent. Thus vaccine research has been a thrust area. Extensive studies have been undertaken in the areas of candidates for vaccine development as well as novel methods for vaccine delivery.

Immunogen source – advancements

Live or attenuated vaccines are considered first and second-generation vaccines respectively. Vaccines comprising DNA plasmids that are capable to express major pathogenic protein sequences in the host are considered third-generation vaccines [3] Though DNA vaccines were found to deliver outstanding immunogenicity with the capacity to be in the host cell for months together, there were concerns of developing tolerance and autoimmune reactions which required an extensive study to prove either way. To prove the strategy as effective it required a clinical trial with a huge number of human volunteers which is an actual risk. Thereby nowadays, the focus shifted to other strategies to discover and develop vaccines [4]. A recent

phase in the advancement of vaccines is the research in the field of epitope vaccines. The parts of amino acid sequences of whole antigens that are responsible for the stimulation of immunogenic responses are known as epitopes or antigenic determinants. Generally, the epitopes are recognized from antibodies, B cells, and T cells. Accurate knowledge of immunogenic protein is required for the development of epitope vaccine. The reverse vaccinology approach was used to derive the information of the codon sequence of the DNA for the development of complementary DNA and further, it will be translated into the protein sequence of the respective protein [5].

Immunoinformatics

With theacquaintance of the primary sequence of antigenic protein, and determining immunogenicity experimentally by using smaller parts of the proteins on animals epitopes can be recognized. As this work is more tedious and expensive, *in silico* prediction programs can be used. This approach led to the development of a new field called immunoinformatics, which involves the analysis of immunological data using various algorithmic models. Various approaches of immunoinformatics are sequence-based methods and structure-based approaches. The first one comprises of various methods like Motif search-based approach, prediction by the artificial neural network, prediction by the support vector machine, Hidden Markov models, prediction by quantitative matrices driven methods involving quantum mechanics. Sequence-based approaches cover docking of peptides and screening of peptide libraries, application of threading algorithms, binding energy, and molecular dynamics [6]. In contrast to the above, the development of B cell epitopes is more challenging because they are discontinuous and are constitutedby amino acid residues that are positioned in diverse regions of the protein and quaternary/tertiary structure of protein formed due to folding forms a region called as epitope. These amino acid groups cannot be isolated as such in the form of an antigen. Hence, Structural Based Reverse Vaccinology (SBRV) which focuses on the use of a monoclonal antibody against the protein antigen to mimic the structure is used for development of epitope vaccine. As part of this, many scientists tried to use machine learning and this strategy was not that successful [5].

Immunotherapy

Vaccines in general modulate immune response. This principle of modulating the body's immune system has been extended towards the therapy of non –infective clinical indicationsand is called immunotherapy. In Immunotherapy or biological therapy, the immune system is either activated or suppressed andrespectively termed as activation immunotherapy and suppression immunotherapy. Cancer immunotherapy and allergen immunotherapy are being widely researched. Immunotherapy has various approaches based on the biological entity being employed e.g. interleukins, cytokines, chemokines, etc. Allergen immunotherapy involves repeated administration of allergens to allergic individuals over a long period to confer long-term relief of symptoms and improvement in the quality of life during subsequent natural allergen exposure [7]. In immunotherapy of cancer, the host's immune response is activated. It considers the activation of host's suppressor mechanisms by modulating checkpoints. It also efforts towards increasing the effector cell number and the production of soluble mediators [8].

Artificial intelligence for vaccine development

Artificial Intelligence (AI) tools have penetrated all realms of scientific research. For vaccine development to AI is being applied. The Human Immunomics Initiative (HII) project from the Harvard T.H. Chan School of Public Health and the Human Vaccines Project together are designing the AI platform for capturing data about the method to generate an immunogenic response and for predicting the responses of the population who are expected to be vaccinated for a range of diseases [9]. Currently, AI is being applied to develop the vaccine for Covid-19. As per the report, development of a computer model that could screen and eliminate 95% of potential candidates and arrive at choices to construct a multi-epitope vaccine, which can attack the spike proteins of coronavirus [10].

Veterinary vaccines

Vaccination for animals is an equally growing area of work especially for the case of zoonoses to prevent harm to humans from infections originating in animals. Viral disease – the SARS-CoV and MERS-CoV have been reported to have originated in bats. It was concluded from studies that SARS-CoV had spread from infected civets to people, while MERS-CoV spreads from infected dromedary camels to humans [11]. Regarding SARS- CoV-2, the latest information directs towards bats being a source of infection [12]. Poultry, cattle, horse, dogs, and cats are a few animals to mention where vaccination is a concern and research advancement is a continued need.

Vaccination delivery advancements

Most of current vaccines are delivered in the form of injection. Injectable vaccines are well established for desired therapeutic outcome and their production facility is geared up to meet global demand. Yet, modes of alternate delivery are desirable to avoid the inconvenience associated with injections. Oral vaccines are commercially available and the oral polio vaccine being the most popular. Oral vaccines are also preferred for immunization for the wildlife population for dreadful diseases such as rabies. Some commercial oral vaccines include LUIVAC (respiratory infection), Vaxchora (cholera), Vivotif, (typhoid), RABORAL V-RG (rabies), Dukoral (Cholera, diarrhea), Tetramune, Rotateq, Rotashield, (Rotavirus), Biostim (Klebsiella) [13].

Mucosal vaccination and vaccine formulations

Pathogens gain access to the body via mucosal sites which is the first line of defence for the body. Mucosal vaccination is an upcoming area for generating protective immunity at mucosal sites and countering pathogen invasion and dissemination. The routes forthe mucosal vaccine are predominantly oral and nasal administration while vaginal, rectal, ocular, and sublingual routes are less popular. The antigenic protein

may be isolated from the infective microbe and formulated for delivery. Reports on mucosal vaccination either as microparticles(PLG particles loaded with ova albumin [14]), nanoparticles (plasmid DNA loaded chitosan nanoparticles for nasal delivery [15]) liposomes(mannose-PEG-cholesterol (MPC)/lipid A-liposomes (MLLs) entrapping model antigen Bovine Serum Albumin (BSA) [16], etc.

Biological carriers for vaccines

Bacteria- Gram-negative (*E .coli* and *S. Typhimurium* as attenuated organisms) and gram-positive bacteria (Lactococcus, Streptococcus, and Lactobacillus) have been used for the vaccine of plasmid DNA as theimmunogenin poultry for mucosal vaccine delivery [17]. Virosomes are another class of carrier system. They are not the virus but are they are unilamellarlipid membrane vesicles made of viral membrane protein and devoid of viral genetic material. The antigenic material is encapsulated in the vesicle for delivery [18].

Plant-based edible vaccines

This vaccination strategy adds to the mucosal vaccination domain of conferring immunity in addition to systemic immunity. Plant-based vaccine development has also been termed molecular farming since the required biopharmaceutical molecules are obtained from genetically engineered plants. The antigenic moiety is protected within the plant cell and need not be specifically harvested and purified. Lettuce leaves containing soluble antigen of *Bacillus anthracis* were developed. These were reported to be stable at room temperature. The antigen did not degrade. Other plants include tomato, papaya, and carrot. The article also reports the use of insects and yeast as source delivery tools for vaccines. The products are in the pathway of clinical trials phase 1 or phase 2 [19].

Sublingual immunotherapy(SLIT)

Allergen immunotherapy has so far been carried out by Subcutaneous (SC) injections. The SC therapy was not favorable since there were fatalities reported. SLIT ispromising especiallyfor allergic rhinitis and asthma [20]. SLIT may be delivered as a tablet, film/strip, or as drops [21].

Other formulation strategies for vaccine delivery

In addition to the above advancements in vaccine delivery, other formulation strategies for vaccines include micellar delivery systems, polymeric nanoparticles, dendrimer based delivery, needle-free delivery, microneedle system for vaccines [18].

Thermostable vaccines- spray drying and other approaches

Spray drying technology has been tailored to meetthe requirements of the vaccine industry. Many vaccines are unstable as liquids or need to be processed by a cold chain management system. A feasible alternate is provided by developing heat-stable vaccines. Virosomes have been processed by spray drying [22]. Similarly for tuberculosis lyophilization of oil-in-water nano emulsion adjuvants has been worked upon to yield stable product [23]. Human papillomavirus capsomere has been delivered as heat-stable microparticles. Glass-forming polymers and trehalose are the first spray-dried antigen/adjuvant formulations to form glassy microparticles. Atomic Layer Deposition (ALD) reactions conducted in fluidized beds are then used to coat the microparticles with defined numbers of molecular layers of alumina that modulate the timed release of the internalized antigen and act as adjuvants [24]. Microneedle array patch technology has been developed for Hepatitis B surface Antigen (HBsAg) employing hydroxyethyl starch (HES) and Chondroitin Sulfate (CS) and saponin QS-21 as an adjuvant, both incorporated in HES-based MNAP [25]

Adjuvant advancements in vaccine development

Adjuvants are substances used to potentiate the immunogenic effect of the vaccine. They also serve other purposes. Different kinds of adjuvants are being looked into for conferring better safety, stability, and efficacy of the antigen. Aluminum hydroxide is a popular adjuvant. It has been reported to protect antigens from degradation [26]. Protein-based adjuvants are potential candidates. Heat-labile toxin produced by the double-mutant *E. coli* is a novel protein adjuvant that is in the preclinical stage with various vaccine candidates [27]. Another protein-based adjuvant reported for mucosal vaccination is Unlipidated outer membrane protein 19 (U-Omp19) can be used in vaccine formulations. U-Omp19 is reported to be able to inhibit gastrointestinal and lysosomal peptidases, and prevent digestion of antigenic protein and enhance their availability to the antigen presenting cells *in vivo* [28]. Polymeric Systems is another area to explore as a vaccine adjuvant. Biodegradable polyanhydrides have been reported to optimally achieve many parameters like enhancing antigenic response, induce immunologic memory, provide better patient acceptance, reduce inflammation, and be stable in challenging environments and thus support the global market [29]. Genetic molecular adjuvants are used to potentiate the response to DNA vaccines. Various plasmids like cytokine encoded, chemokine encoded, co-stimulatory molecule encoded plasmids, and plasmids encoding pathogen-Recognition Receptor (PRR) Ligands and Immune-Signaling Molecules are certain molecular adjuvants [30].

Future prospects

Nowadays, nucleic acid vaccines are becoming more promising as they are able to elicit both cell mediated as well as humoral immunity. An effort has been done for the use of nanoparticles for nucleic acid vaccine delivery to overcome the action of endonucleases on vaccines when given in injection form. Use of liposomal nanoparticles, polymeric nanoparticle system, natural polymers, inorganic nanoparticles, peptide based nanoparticles was done to deliver nucleic acid vaccines and many formulations are in different phases of clinical trials [31]. Attempts were done for topical delivery of vaccines through the use of star shaped, milli meter scaled, made of aluminium oxide or stainless steel possessing micron scale projections which are able to make pores across stratum corneum. Though, it was not successful as expected, and most challenge, this

new technology require further research to use in its fullest potential [32]. Even various vectors like, influenza A virus vector, Venezuelan Equine Encephalitis (VEE) virus vector, adenovirus-based vectors, lactic acid bacterial vectors were tried to deliver vaccines against botulism, but needs further optimization [33]. Orotransmucosal delivery of vaccines using various hydrogels in different composition accompanied with various artificial intelligence tools [34].

The advancements in vaccine technology both in the candidates suitable for generating an immunogenic response as well as in delivery of the vaccine mentioned are potential areas for exploration and commercialization. In the current wake of Covid-19 there has been global uproar in vaccine research, clinical trials, approval, large scale manufacturing, cold chain management and mass vaccination schemes. The possible mutations and the efficacy of the vaccines on the new strains is debatable. Thus if the existing AI tools may be used to predict possible mutations in the microbes/virus there would be better chance to design and develop better vaccines. Similarly AI can be used with the VARES – vaccine adverse event report system to work towards selecting population to whom vaccine should not be administered and thus may be avoid fatalities.

Conclusion

In the present-day situation, vaccines are the most useful biological products for the treatment of various infectious disorders. The efficacy, specificity and ability of vaccines to alter immune response has made them to be in prime focus because more active immune response against any particular disease is more effective than the small molecules which are not free from side effects. Era of artificial intelligence has prompted scientist to gather and analyse the available data for the use of vaccine development. Though technological advancements intervened in the vaccine development, attempts are made to improve formulation and vaccine delivery methodologies, to overcome the limitations of efficacy, selectivity and ability to stimulate immune response. In summary, vaccine designing, development, formulation and delivery need to go hand in hand to reap the advantage of vaccines so that mankind will get its advantage in its fullest form.

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