

Review Article

Vaccination: A Future Perspective

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Abstract | Vaccines have always drawn attention when it comes to tackling diverse era of infectious and non-infectious diseases. Since the gradual emergence of the concept of vaccines and their epochal success in control, prevention and eradication of many diseases, researchers have always seen immense potential in the field of immunoprophylaxis to combat the growing demands of health sector. With new emerging diseases to confront and strategic change in host invasion and immune evasion by microbes augments the need to transform vaccines with a more futuristic perspective. Researchers foresee to revolutionize the modern vaccination era by in-depth understanding of immune system and bringing about transformation in every component of vaccine formulation (like antigen, adjuvant etc.) by amalgamating other fields like recombinant DNA technology, molecular genetics, cellular immunology, structural biology, bioinformatics, computational biology, nanotechnology, formulation technologies and systems biology. In order to cope up with the changing scenario of vaccine industry, the knowledge of the existing trend in this field becomes indispensable. Here we highlight the existing and futuristic approach towards vaccines which will keep veterinarians, researchers and clinicians in other sectors abreast with the technologies and hasten research in this direction.

Keywords | Vaccine, Future, Adjuvant

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INTRODUCTION

Over the past years the field of vaccinology has undergone a substantial transformation (Flingai et al., 2013). Where earlier, civilisations were being wiped out due to unavailability of protection against certain diseases like plague, now it is being possible to eradicate diseases like small pox from the globe and control many dreaded diseases of human and veterinary significance (Kanampalliwar et al., 2013). There

has been a shift in technology of production of vaccine candidate from physical and chemical inactivation of microorganisms to the production of recombinant microbes, deletion mutants and expression of putative proteins etc. The field of vaccinology is undergoing a rapid transformation by the gradual understanding and incorporation of recombinant DNA technology, molecular genetics, cellular immunology, structural biology, bioinformatics, computational biology, nanotechnology, formulation technologies and

systems biology. More recently, new methods of antigen discovery and design as well as investigation of vaccine responses have been applied, including reverse vaccinology (Rinaudo et al., 2009).

While the development and widespread use of effective vaccines has an extraordinary impact on global health, there remain many infectious and other diseases for which vaccines are not available. In general, the viruses, bacteria, and parasites for which new vaccines are needed are either complex in their pathogenesis, exhibit extensive variability, or have evolved evasive mechanisms to bypass the immune system. The development of vaccines for many pathogens remains elusive, and there is a growing requirement for the fast development of effective vaccines for emerging diseases (Morens et al., 2008). Here, we discuss the technological advances that are propelling into a new era in vaccinology, highlighting the various aspects which remain the target of development, for combating the challenges of emerging infectious and non-infectious diseases. The cognition of gradual shift from the conventional vaccination strategy towards a more calculated and novel approach will not only keep human and animal sector abreast of the current trends in immunoprophylaxis but will also encourage research in a direction that will improve preparedness for coping up with the upcoming demands of the health sector.

A BRIEF HISTORY

Vaccination surfaced as a result of cautiously planned attempt of humans in order to shield themselves from infectious diseases centuries ago when Indian Buddhist monks drank snake venom to confer immunity to snake bite. The Greek historian Thucydides in 429 BC observed that those who survived the plague in Athens did not become re-infected with the disease and from there the concept of immunity was born. In the eleventh century the Chinese insufflated variola scabs into nose to confer resistance against small pox, which was based on the observation of protection by prior exposure to the disease (Plotkin, 2005). Lady Mary Montague, wife of the English ambassador at Istanbul, introduced the practise of variolation in England. The common observation that milkmaids were generally immune to smallpox was the impulse for Jenner's epochal hypothesis of prevention of small pox by exposure to cow pox, which was proved by inoculating the subject with pus in the blisters from

cowpox of milkmaids and subsequent challenge with the virus. This discovery not only led to the eradication of small pox in the twentieth century, but also established the idea of developing immunity against an organism by prior exposure to it.

The illustration of 'chance & prepared of mind' could be more appropriately derived from Pasteur's accidental discovery of attenuation in his own laboratory by ageing of a culture of fowl cholera. Based on this observation, Pasteur hypothesised the attenuation of pathogens by environmental and chemical insults and confirmed it by inventing vaccines for fowl cholera, anthrax and rabies (Plotkin, 2005). With the advent of time, the technique of attenuation by passaging organisms in artificial media and in unnatural host was a major breakthrough by Calmette and Guérin and Theiler, who used the methodology to attenuate *Mycobacterium bovis* and yellow fever virus respectively. The method of attenuating viral vaccines by propagating in cell culture scouted towards the development of several vaccines, including polio, measles, mumps, and rubella (Koff et al., 2013). Meanwhile, the concept of humeral and cellular basis of immunity was established, which provided a more precise approach towards regulation of immune response.

CONVENTIONAL VACCINATION STRATEGY

Pasteur's early approaches to vaccine development, even today are the two yardsticks of vaccine technology. Existing vaccines can be divided into two broad groups: live and killed vaccines. Live vaccines induce immunity by mimicking natural infections using attenuated forms of pathogens while killed vaccines use the whole pathogen or their components to induce protective immunity. Conventional approach of vaccine production are mostly based on the cultivation of the microorganism in laboratory conditions followed isolation of individual components in pure form, which are finally tested for its immunogenicity with the exception to non-cultivable microorganisms. In many cases the predicted/assumed antigens expressed during natural infections fail to be expressed in laboratory conditions. Moreover, it has been observed that the proteins which are most abundant and easily purified may not provide adequate protection. (Capechi et al., 2004).

The use of sequence data of microorganisms and bioinformatics tools for the design of vaccines is a recent approach towards antigen discovery and has been termed “Reverse vaccinology”. By taking into focus the whole genome sequences, current computer programs allow predicting the function or the putative cellular localization of the newly-identified open reading frames (ORFs) thus providing a virtual catalogue of all the candidate molecules which can be used as an effective vaccine. Moreover the genomic information is also used along with the amalgamation of “Functional Genomics” like in vivo expression technology (IVET), signature tagged mutagenesis (STM), DNA microarrays and proteomics (two-dimensional gel electrophoresis and mass spectrometry) in order to select novel antigens (Capechi et al., 2004). Besides reverse vaccinology, “antigenome analysis” using libraries of genetically expressed antigens and screening for immunogenicity of the proteins during infection, has also evolved (Giefing et al., 2008). Even the gradual evolution in mass spectrometry analysis has enabled direct quantification of bacterial surface antigens (Koff et al., 2013). Though, the aforementioned methods have enormous capability of identifying and tapping potential antigens for inclusion in vaccines, they remain limited in their capacity to predict antigens with protective ability. Efforts have also been made towards identification of subjects with broadly neutralizing antibody (bnAbs) serum responses, determination of the structure of the binding sites of bnAbs and mimicking the epitopic binding sites of such bnAbs by exploiting the memory cell repertoire of infected individual, for immunogens to elicit such bnAbs (Simek et al., 2009; Walker et al., 2009; Kwong et al., 2012; Burton et al., 2012; Zhou et al., 2010).

PROTEOMICS IN VACCINE DESIGN

With the accessibility to genomic sequence data, advances in mass spectrometry analysis and an innumerable ways of characterizing proteins have made it possible to separate and identify proteins expressed in a cell. The entire set of proteins encoded by the genome has been defined as “proteome” (Grandi, 2001). In proteome analysis, a protein mixture is first resolved leading to the separation of individual components followed by proteolytic digestion with specific peptides to deliver discrete peptides. The peptides are

evaluated by mass spectrometry and the experimental value is compared with all the predicted ones, thus ascertaining the product of a specific gene. (Capechi et al., 2004). Analysis of the proteome allows identification of proteins under different growth conditions. This approach has been used extensively to identify novel vaccine candidates against several pathogens.

ADJUVANTS & MODERN DELIVERY TECHNOLOGIES

Adjuvants are molecules, potentiating the efficacy and longevity of specific immune response to antigens, with minimal toxicity and adverse effects (Wack et al., 2005). The addition of adjuvants to vaccines not only enhances the immune response, but also reduces the antigen concentration and dosage, effectively directing appropriate immune response. An effective adjuvant formulation utilizes multiple components and methodologies to achieve the desired immunological response (Schijns, 2000). Of the various mechanisms used, the most effective ones includes establishing antigen depots, enhancing antigen presentation by dendritic cells (DC) to effector cells like CD8+ cytotoxic T-lymphocyte (CTL) or CD4+ T-helper (Th) lymphocyte responses (Mohan et al., 2013). Adjuvants may also exert their activities by targeted distribution of antigen to specific cells, improving stability of antigen and by acting as immune modulators as in case of microbial and endogenous adjuvants (Dangi et al., 2011). Realizing the need of a powerful adjuvant, Freund in the mid-1930s, developed a powerful immunologic adjuvant known as Freund’s complete adjuvant (FCA) basically composed of a water-in-mineral oil emulsion containing killed mycobacteria. But today the adjuvant technology has transcended way beyond in search of a complete adjuvant, so as to revolutionize the modern era of vaccination. An adjuvant can be basically divided into two classes: delivery systems and immunopotentiators, mostly basing on their mechanism of action (Dangi et al., 2011). Immunostimulatory/ Immunopotentiating adjuvant act predominantly at the cytokine level or by activation of co-stimulatory signals, with due emphasis on the type of antigen used for optimal immune response (Mbawuiké et al., 2007). However, on a different note, delivery systems emphasize on the presentation, protection and targeted delivery of the antigen. A brief description of some of the potential adjuvants which are/can be used in various vaccine formulations to

achieve specific immune response through desired pathway has been described below.

Aluminium salts such as aluminium hydroxide, aluminium phosphate and potassium aluminium sulphate (alum) work by inducing inflammation and antigen retention at the injection site for a prolonged period thus allowing detection by the immune cells (Glenny et al., 1926). However, aluminium containing vaccines may be associated with local reactions. Liposomes on the other hand are vesicles in which an aqueous core is encapsulated within one or more phospholipid bilayers. Liposomes are highly flexible delivery systems, able to carry both hydrophobic and hydrophilic substances. They are relatively non-toxic and can be conjugated to antibodies or ligands and can be engineered for vaccine delivery, either by core or surface (Mohanty et al., 2014). Similarly, viral vectored vaccines are also delivery systems but differ from liposomes in the manner that viral-vectored vaccines consist of a non-replicating virus that contains genetic material from a pathogen to which specific immunity is desired. Such vectored vaccines stimulate both mucosal and systemic immunity and are commonly referred to as live recombinant vaccines (Plotkin, 2005). Adenovirus, which has been administered orally as its own vaccine for decades, has also provided a frequent vector platform for many of these types of vaccines, including delivery systems for tetanus (Shi et al., 2001) and influenza (Van Kampen et al., 2005; Vemula and Mittal, 2010).

VLP (Virus like Particle) are essentially non-infective viruses with self-assembled viral envelope proteins without the accompanying genetic material. In this system the envelope of one virus is used as a framework to which additional components of the virus or another virus or pathogen are attached or inserted (Metcalf et al., 2006). Both types of particles maintain morphology and cell penetrating ability similar to infective viral particles (Huckriede et al., 2005) and are capable of inciting both humoral and cell mediated immunity (Grgacic et al., 2006). Bacterial components are also being used in adjuvant formulations to enhance the immune response through various pathogen recognition receptors (PRR). Likewise Monophosphoryl lipid A is a TLR-4 receptor agonist (Persing et al., 2006) derived by detoxifying lipopolysaccharide (LPS) from *Salmonella minnesota* R595. After detoxification, the resulting MPL maintains

adjuvacy and is a versatile vaccine adjuvant which in combination with other adjuvant systems can deliver more dynamic response (Baldrige et al., 1999). MPL has been shown to include cytokine release leading to activation of immune cells (Neuzil et al., 2006). Similarly CpG motifs are six deoxynucleotides-long DNA sequences with a central CpG dinucleotide, and are more frequent in bacterial DNA than that of mammalian DNA. CpG motifs are recognized by the Toll like receptor (TLR 9) (Cornelie et al., 2004) inducing the secretion of type I interferons and IL-12 by cells of the innate immune system, thus triggering Th1 cellular response. Therefore, CpG-containing DNA-based molecules have been advocated for therapeutic applications and also for adjuvanting other types of vaccines (Klinman, 2004).

Nowadays, a wide number of adjuvants are squalene based formulations. Squalene is a linear triterpene that is used mostly because of its stability and biocompatibility and is extensively utilized in parenteral emulsions for drug and vaccine delivery. Emulsions containing squalene facilitate solubilisation, enhanced release, and cell uptake of target molecules. Squalene and its hydrogenated form, squalane, both have been ideally suited for making stable and non-toxic emulsions. Combination adjuvant known as DETOX™ contains MPL® and *Mycobacterium phlei* cell wall skeletons in a squalene emulsion and has been used in the clinical case of melanoma (Mitchell et al., 1988) and ovarian cancer (MacLean et al., 1992). Such MPL®-containing vaccine formulations have been found to enhance both cellular and humoral immune responses with minimal toxicity (depending on the antigen) relative to non-MPL® formulations (Mitchell et al., 1988). A formulation known as MF59, which is an oil in water emulsion, squalene based vaccine adjuvant also significantly enhances immune response to a wide variety of antigens (Brito et al., 2011). However it has been observed to cause serious autoimmune disorders and hence seldom used in human vaccination except influenza vaccine. However in a more convenient approach Montanide™, including ISA 50V, 51, 206 and 720 which are either water-in-oil or oil-in-water emulsions are also used in strategic vaccine formulations. ISA 206 and 50V have been used only in veterinary vaccine formulations while the others are under investigation for use in humans (Aucouturie et al., 2002). The immune enhancement produced by the Montanide™ emulsions is believed to be due to the

formation of a depot at the site of injection (Miles et al., 2005). These emulsions are under clinical trials for vaccines against malaria and various cancers (Miles et al., 2005; Toledo et al., 2001).

ROUTES OF VACCINATION

Another vital aspect of vaccination is the route by which the formulations have to be delivered, without the proper channel the designed engineered vaccine formulation will not meet the targeted ends, irrespective of the generation to which it may belong. Traditional vaccine administration methodologies are limited by their nature of inducing local reaction, inconvenience, safety and cost effectiveness. Very few vaccines are being administered intranasal, orally and subcutaneously for diseases like influenza, polio, and measles-mumps-rubella respectively (Plotkin, 2005). Ongoing research on alternative experimental administration strategies includes ballistic delivery to skin (the gene gun), the transdermal patch and other intradermal methods, plus sublingual, aerosol, rectal and vaginal mucosal vaccines. The main advantages of alternative delivery strategies are the potential to induce immune responses at the common portals of pathogen entry (e. g. oral polio vaccine replicating in the gut), convenience (e. g. ease of use of the transdermal patch), combination of vaccines to reduce or simplify the vaccination schedule, and reduction or elimination of administration via standard hypodermic needle injection. Despite the intuitive value of these approaches, few vaccines today are administered via non-IM routes. This is for several reasons including feasibility, lack of proven efficacy and limited safety data. (Stanberry and Strugnell, 2011).

The inconvenience and apprehensions of a needle piercing the body is prevalent in both animal and human health care sector. The closest development in this area is the transdermal application (Matyas et al., 2004). Many devices have been developed to deliver antigens across the skin by means of patches infused with adjuvant applied to mild skin abrasion using micro-needles to pierce the stratum corneum. Once past the superficial layer, it enhances contact with dendritic cells, thus potentiating immune response (Plotkin, 2005).

Till date the concept of immunization used to encompass only infectious diseases but in near future goals are being set for targeting non infectious diseases like cancer, and desensitization methods of tackling allergies with better antigens for inducing IgG rather than IgE antibodies are in development (Linhart et al., 2012). It is also intriguing that individuals with inherited mutations that predispose to cancer might be immunized prophylactically before cancer develops. Attempts are also being made towards tolerance to auto antigens (Larsson et al., 2011), contraception by immunization against hormones (Vizcarra et al., 2012) combating multiple ailment by immunization against cholesterol (Fattori et al., 2012) and antibody mediated clearance of drugs (Kinsey, 2014). Moreover the use of mice models to predict and study the immune response has emerged with various setbacks of difference in dosage as compared to animals and humans and even the minor variations in inciting an immune response in mice will have significant impact in predicting the effect in higher targets. So efforts have been made towards development of humanized mice which are very promising in mimicking human immune response are being validated (Koff et al., 2013).

CONCLUSION

Despite the immense advancements made in the field of vaccinology there are many puzzles yet to be deciphered, also many challenges keep popping up as we go deep in search of solutions. Moreover the lack of understanding of host-pathogen interaction, immunological responses under varying conditions and the inability of fine tuning protective response with antigenic variability is irrefutable. The radical changes in this field has to be incorporated in both human and animal sectors irrespective of the targeted aetiology, as the basic fundamental methodology of formulation and devising of an effective vaccine strategy remains the same. The ongoing project against major global killers such as AIDS, tuberculosis, malaria, and other infectious diseases should revolutionise the health sector as a whole thus emphasizing more on successful vaccine development against allergies, autoimmune diseases, disease of zoonotic importance and cancers; and provide a novel vaccines against new and emerging diseases.

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