AN OVERVIEW OF RECOMBINANT VACCINE TECHNOLOGY, ADJUVANTS AND VACCINE DELIVERY METHODS

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ABSTRACT

Development of an effective vaccine is of paramount important in disease prevention and control. As such, recombinant technology can serve as a gateway for the development of safe and effective vaccines that can be delivered effectively with an appropriate adjuvant. Therefore, this paper aimed to review the role of recombinant vaccine technology, new adjuvants and the challenge of vaccine delivery. Related peer-reviewed journal article searches were conducted using a subscribed database at the Universiti Putra Malaysia library, involving areas of Health Sciences and Medicine via Medline, SCOPUS and Google Scholar. New generation vaccines include highly purified synthetic or recombinant antigens that stimulate effective cell-mediated immune and mucosal immunity. In order to enhance their efficacy, a number of adjuvants are used. Efforts have also been made to explore the usage of non-invasive routes of administration, devices and equipment for optimized antigen and immune-potentiator delivery of the immune system. Recombinant vaccine technology is rapid, compared to the traditional method of vaccine development and does not require the handling of live viruses. It is, therefore, a promising technology for developing a future vaccine to curb emerging and re-emerging viral infections that may be life-threatening or teratogenic.

Keywords: Vaccine adjuvant, Vaccine delivery, Immunity, Recombinant vaccine

INTRODUCTION

The most important driving force for effective vaccine development is an advancement in the fields of science and medicine. Pasture, Ramon, Merieux and Koch established the germ theory and developed killed (inactivated) or live attenuated pathogen vaccines, signaling the first golden age of vaccines [1]. The second golden age in vaccine development was a result of advances in cell culture, technology, which took place in the mid-20th century [2]. These revolutions in cell culture allowed for the development of inactivated vaccines to effectively prevent hepatitis A, as well as live attenuated vaccines against *mumps, measles, polio, varicella* and *rotavirus*. Vaccine delivery is a crucial aspect that needs to be addressed in term of the challenges associated with vaccine development, such as the route of vaccine administration and antigen delivery and activation of the relevant arms of the immune system [3]. Vaccines are formulated for administration for a specific part of the body based on the type of immunity that needs to be induced in order to have effective protection.

The most common routes of vaccine administration are intramuscular and subcutaneous. However, needle-free vaccines are currently advocated, such as oral vaccines that aim to induce mucosal immunity, aerosol (inhalational) vaccines [4], needle-free injections [5], nanoparticle-mediated transcutaneous needle-free vaccine delivery via hair follicles [6], skin patches [7], and edible vaccines [8]. These alternative routes of vaccine administration might increase the willingness of the people to be vaccinated given the easier and more convenient route of administration, thereby increasing vaccine coverage. The used of these alternative routes will, in addition, affect the quality of the immune response. For instance, oral vaccination will increase mucosal immunity; most pathogens get access to the body through mucosal surfaces, so there is a need for an effective vaccine that will protect mucous membranes. Hence, knowledge of recombinant vaccine technology, new adjuvants and vaccine delivery methods are of optimal importance.

Literature search

Peer-reviewed journal article searches were conducted using a subscribed database at the Universiti Putra Malaysia library, involving areas of Health Sciences and Medicine via Medline, SCOPUS, and Google Scholar. All searches were limited to, articles published in the last 20 y. All publications were in English, and duplicates, conference abstracts, comments and short communications were sorted and removed. The initial search result gave us 4,695 articles which were screened based on title relevance, leaving 482 full-text review articles, out of which we cited 77 articles in this review.

An overview of vaccine development

After the development of the first smallpox vaccine by Edward Jenner in 1796 [9], the evolution of vaccines continued at a very slow pace until several decades ago with scientific breakthroughs and the discovery of new technologies, leading to rapid advances in virology, molecular biology, and vaccinology. The first generation vaccines were somewhat crude, consisting of partially purified attenuated virus, as in rabies and smallpox vaccines, or inactivated bacteria as in the vaccine for *pertussis*. As time passed, more refined methods were developed, such as virus inactivation, as in the case of the hepatitis A vaccine, virus-like synthesis using recombinant technology, as in the *human papilloma virus and hepatitis B* vaccines, and the purification of polysaccharides, as in pneumococcal vaccines. Therefore, vaccines are described based on these methods of development, i.e. as live attenuated, inactivated or killed, or recombinant vaccine, as shown in table 1.

Types of vaccines

Live attenuated vaccines

This type of vaccine is made of a whole replication-competent microorganism attenuated in pathogenicity. It generally generates potent, long-lasting protection with fewer inoculations. However, there are potential safety risks to immune compromised recipients because the pathogen can revert back to a virulent state. Moreover, the vaccine may be neutralized by maternal antibodies and enhance the spread of mutations. The bacillus Calmette Guérin (BCG) vaccine against tuberculosis (TB), the measles, mumps and rubella vaccine (MMR), and the polio vaccine are examples of attenuated live vaccines. There is a need for a new approach in vaccine development that will move away

from the inactivated or live-attenuated vaccine approach toward the safer subunit vaccine approach. To achieve this, there has been tremendous development over the past two to three decades in the field of vaccinology as a result of recombinant technology, which solved the problems of the pathogen converting back to a virulent form and neutralization by maternal antibodies. The recombinant Mycobacterium tuberculosis vaccine was developed using recombinant technology to achieve stronger protective efficacy and increased safety [28, 41, 42].

A substance must be able to withstand the challenges of efficacy, scalability, reproducibility and biocompatibility in order to serve as a potential vaccine adjuvant. Biodegradable polymers have the ability to withstand these challenges and are considered promising candidates for next generation platform vaccine development. Additionally, many polymeric vaccine adjuvants (table 2) have been found to deliver the vaccine successfully, target the immune system and effectively elicit the required immune response [43-47].

Table 1: A list of selected approved vaccines for use in humans and their types *Vaccine licensed, **Vaccine developed

Organism or pathogen	Type	License, or developed	Reference
Virus			
Adenovirus 4 and 7	Live attenuated	2011*	[10]
Influenza (nasal spray)	Live attenuated	2009*	[11]
Measles	Live attenuated	1963*	[12]
Mums	Live attenuated	1967*	[13]
Polio Sabin (OPV)	Live attenuated	1960*	[14]
Rotavirus	Live attenuated	1998*	[15]
Rotavirus pentavalent	Live attenuated	2006*	[16]
Rubella	Live attenuated	1969*	[17]
Varicella zoster	Live attenuated	1995*	[18]
Yellow fever	Live attenuated	1953*	[19]
Hepatitis A	Inactivated or killed	1995*	[20]
Hepatitis B	Inactivated or killed	1981*	[21]
Influenza (injection)	Inactivated or killed	1945*	[22]
Japanese encephalitis	Inactivated or killed	1992*	[23]
Polio Salk (IPV)	Inactivated or killed	1955*	[24]
Hepatitis B	Recombinant	1986*	[25]
Human papilloma quadrivalent	Recombinant	2009*	[26]
Influenza	Recombinant	2013*	[27]
Bacterium			
Tuberculosis	Live attenuated	1927**	[28]
Typhoid	Live attenuated	1896**	[29]
Anthrax	Inactivated or killed	1970*	[30]
Cerebro-spinal meningitis	Inactivated or killed	1975*	[31]
Cholera	Inactivated or killed	1896**	[32]
Pertussis	Inactivated or killed	1918*	[33]
Plague	Inactivated or killed	1897**	[34]
Rabies	Inactivated or killed	1970*	[35]
Diphtheria	Toxoid	1923**	[36]
Tetanus	Toxoid	1927**	[37]
BCG	Recombinant	1990**	[38]
Meningococcal	Recombinant	2014*	[39]
Pneumococcal 13-valent	Conjugate	2010*	[40]

OPV (oral polio vaccine); BCG (Bacillus Calmette–Guérin); IPV (Inactivated polio vaccine)

Table 2: List of some commonly used adjuvants approved for human vaccines

Conventional adjuvants	Polymeric adjuvants
Mineral salts (*aluminium* salts)	*Chitosan*
Emulsions (Freund's adjuvant)	Naturally occurring/derived polymers
Immune stimulatory complexes (ISCOMs)	*Starch*
Microorganism-derived adjuvants	*Alginate*
Cytokines	Synthetic polymers
Virosomes and virus-like particles	*Dextran*
	Polystyrene
	Polyesters
	Amphiphilic block copolymers
	Polyanhydrides

Killed vaccines

These vaccines are composed of a whole replication-incompetent microorganism; therefore they are safer than live vaccines and have a longer shelf life. However, these vaccines lack the potency of live vaccines because they do not elicit an effective cell-mediated immune response and induce poor mucosal immunity. This is because they can easily be cleared from the body due to the lack of replication, but they also give rise to a greater inflammatory response than the newer subunit vaccines because most of the pathogenic components are preserved. The absence of safety data on the killed whole-cell oral cholera (*RBS-WC*) vaccine is of great concern, as well as the lack of expected adverse events from a killed oral bacterial vaccine. A live oral recombinant cholera vaccine was developed from a non-toxigenic *Vibrio cholera* O1 El Tor strain using ctxB gene insertion [48]. The vaccine has been found to be safe and immunogenic in adults in a cholera endemic region, and no additional benefit after two doses was seen in a clinical trial.

The majority of killed vaccines in humans was formulated with *aluminium phosphate* [49]. Unfortunately, this produces a skewed immune response that favours systemic antibody production and gives little mucosal or cellular immunity, which are required for the effective control of an infection. The development of safe and effective adjuvants for humans is a hot topic in vaccine research and, as a result, some novel adjuvants such as *MF59, AS01-AS03* and *ISOMS* have been licensed to be used for human vaccines [50].

DNA vaccines

This is a new generation type of vaccine that is attractive based on simplicity and considerable advantages over conventional vaccines. The fundamental principle of DNA vaccination is to induce immunity by transfecting host cells with plasmid DNA encoding the required antigen, contrary to injecting an antigen in the form of a protein or peptide. Once vaccinated with a DNA vaccine, the host cell will produce the antigen, which is encoded by the DNA, thereby inducing immunity against this specific antigen [51, 52]. The most remarkable advantages of DNA vaccines are their low cost, ease of manufacturing, stability at room temperature and their ability to induce both humoral and cellular immune responses [53]. Certainly, this is of great significance in achieving effectiveness in vaccination programs in developing countries due to the fact that no cold chain is required for DNA vaccines, However, to date, no DNA vaccine has been approved for human use, although several clinical trials are being conducted on HIV and some cancers. Some DNA vaccines are approved for veterinary use [54, 55].

Subunit vaccines

These are vaccines that contain one or more part of the pathogen rather than the whole pathogen. As such, they are composed of recombinant protein or peptides, and in some cases polysaccharides that are normally present in the structure of the targeted pathogen [56, 57]. Subunit vaccines have substantial advantages over traditional vaccines in terms of the cost of production and safety, because they made up of highly purified and well-defined components, and lack the ability to replicate, thus avoiding the use of unwanted materials capable of initiating a deleterious host response [58]. In a virus subunit vaccine, split vaccines are the most common form. These vaccines were developed by disrupting the structure of the virus, resulting in a mixture of various components of the virus. As an alternative, subunit vaccines may contain one or more proteins or peptide fragments of viruses or bacteria which, in some cases, might be adequately and independently immunogenic. For instance, the influenza subunit vaccine is composed of two purified antigens (hemagglutinin (HA) and neuraminidase (NA) that are isolated from three seasonal *influenza virus* strains and combined to form a trivalent vaccine, which may or may not contain an adjuvant [59]. Likewise, the hepatitis B vaccine contains the surface antigen (HBsAg) alone, which is sufficiently immunogenic. Similarly, the nonstructural protein of *hepatitis C virus* is of significant potential peptide subunit vaccine [60]. The vaccine based on recombinant HBsAg was the first commercially available genetically engineered vaccine product to be used globally. Nevertheless, in some cases, highly purified subunit proteins are deficient in intrinsic pathogenic features that render the protein-based antigen weakly immunogenic on its own. Thus, an adjuvant is required to facilitate the induction of an effective immune response and potentially modulate the immune response [61, 62].

Bacterial subunit vaccines are of two main types: the toxoid vaccines, which target bacterial infections where toxins are the main cause of disease, and the polysaccharide-based subunit vaccines, targeting infections with encapsulated bacteria. Toxoids are produced by inactivating bacterial toxins by converting them into a detoxified form; this can be achieved by treating the toxin with *formaldehyde*, resulting in a toxoid that can be used safely for vaccination [63]. The immune system neutralizes natural toxins by generating antibodies against the toxoid, achieved as a result of the close resemblance of the toxoid to the toxin. Examples of this type are diphtheria, pertussis and tetanus vaccines. Capsular polysaccharide subunit vaccines include vaccines against *Haemophilus influenza* type B, *Streptococcus pneumoniae* and *Neisseria meningitides* [64]. A variant of this is the conjugate vaccine, which is developed by covalently attaching bacterial polysaccharides (the antigen) to a carrier protein, resulting in a more efficacious vaccine, such as the one against the tetanus toxoid.

Cell-based vaccines

This type of vaccine is developed in mammalian cell lines rather than chicken egg embryos. The method involves growing the virus in mammalian cells or loading antigen into cells, depending on the application, i.e. infection control or cancer prevention, respectively. In terms of cancer, dendritic cells (DCs) are being explored for cell-based vaccines. DCs are antigen processing cells whose function is to acquire, process and present antigens to T cells, providing the cytokines and stimulatory signals required for the induction of T cell proliferation and differentiation into effector cells. In this vaccination strategy, *in vitro* generated dendritic cells (DCs) are loaded with specific antigens and infused into a patient, thereby eliciting T cell-mediated responses against the targeted pathogen. This type of vaccine is mostly used in cancer therapy as the function of DCs is often dampened or destabilized by tumour secreted factors. Although the DC-based vaccines have been shown to prevent tumor progression in animal studies [65, 66], the results of clinical trials in humans have been disappointing, showing only marginal benefits to patients [67]. Therefore, there is still need to improve the stimulatory capacity and efficacy of cell-based vaccines. The improvements have to be significant enough to justify the complexity of manufacturing these vaccines, although strategies to simplify and shorten ex vivo DC-vaccine generation are under investigation.

It is, therefore, important to evaluate the prospect of combining simple DC vaccines with other anticancer drugs, particularly in patients that are yet to generate a spontaneous T cell response against their tumours. Despite early disappointments in cancer prevention, cell-based vaccines have been found to have a place in infectious disease control with the approval of the first cell-based vaccine (*Flucelvax*) by the American Food and Drug Administration on November 20 2012. This vaccine targets three influenza sub-types: influenza A subtypes H1N1 and H3N3, and *influenza* B [68, 69].

Recombinant vaccine technology

This is a modern method of developing vaccines. It involves the recombinant expression of proteins and viral vectors. This technology provides the possibility of developing vaccines against difficult-to-culture or non-culturable viruses and eliminates safety risks by using bioprocesses that are more controlled with defined process components and a shorter process of production, which is very important in terms of responding to a pandemic [70, 71]. A typical example of a recombinant vaccine is *Recombivax*, a hepatitis B recombinant DNA vaccine, which was the first to be licensed. In this vaccine, recombinant hepatitis surface antigen is expressed in a yeast cell (*Saccharomyces cerevisiae*) [25], in contrast to the original vaccine that was made by purifying HBV particles from infected blood [72]. Over the past decade, several new vaccines have been developed using recombinant technology. One common approach is reverse vaccinology, in which genome analysis is performed to identify a repertoire of antigens that are highly antigenic, surface exposed and conserved across multiple strains.

The most immunogenic epitopes are sequenced and evaluated for appropriateness for a vaccine formulation and then patented by a pharmaceutical company for commercialization. Recombinant vaccine technology will be key to future vaccine development considering the current outbreaks of emerging and re-emerging viral infections that are life-threatening and teratogenic, such as the *Lassa virus*, *Ebola virus,* and *Zika virus*. This is because the upstream process in recombinant vaccine technology is fast compared to cell culture and in egg production and does not require the handling of live virus and the accompanying expensive Biosafety containment equipment.

Vaccine adjuvants

The term "adjuvant" is from the Latin word *adjuvare,* meaning to help [73]. As such, vaccine adjuvants can be defined as a component in a vaccine that has the ability to potentiate the immune response to the targeted antigen and modulate it towards a desired immune response. To potentiate the desired immune response, adjuvants employ different mechanisms such as prolonging the presence of antigen in the blood, increased uptake of antigen and presentation to antigen presenting cells (APC), lymphocyte and macrophage activation, and supporting cytokine production [74]. Adjuvants used in human vaccines are of different types, such as cytokines, bacterial products, plant saponins and inorganic compounds like *aluminum salts*, *aluminium phosphate*, *aluminium hydroxide* and *calcium phosphate*.

Aluminium salt (alum) is the most commonly used adjuvant [75]. It was discovered in 1926 by Glenny and has been used in vaccines for more than 70 y [76]; it was the only approved adjuvant for use in human vaccines for many years [77]. The role of *aluminium* as a vaccine adjuvant is mediated by the ability of highly charged *aluminium* particles to absorb antigen [61], thus acting as an immunopotentiator by directly activating the innate immune system via pattern recognition receptors (PRR) and activating APCs [78, 79]. Toll-like receptor (TLRs) agonists have been shown to be promising vaccine adjuvants in both preclinical and clinical studies, as they provide the opportunity to induce distinct cytokine profiles, thereby modulating and tailoring the vaccine-induced immune response [80]. New synthetic TLR agonists are being developed and their availability has expanded [81-83].

To generate the optimal immunological effect, consideration is given to the most appropriate adjuvant for a particular formulation, based on the antigenic component of choice, the desired route of administration, the type of immune response required, the stability of the vaccine, as well as potential side effects [61]. Therefore, for an adjuvant to be added to a vaccine, there must be justification in terms of safety, tolerability and efficacy, and it should be selected based on the risk/benefit ratio and the target population. Substantial progress has been made so far in discovering new, efficient subunit vaccine adjuvants that have been marketed as approved components of licensed vaccines [84-86].

Vaccine delivery systems

Vaccine delivery systems are particulate in nature. The delivery system should be of a similar size as the given pathogen, which is a natural target for APCs, and should aim to effectively deliver vaccine components to target APCs, thereby improving the quantity of antigen reaching these cells and facilitating the induction of the immune response. It is therefore of great importance to combine a delivery system and an immunopotentiator in order to enhance antigen delivery and to stimulate the innate immune system [87, 88]. The delivery system can potentially control antigen dynamics and kinetics by protecting the antigen from degradation, delaying clearance of the antigen from the site of injection, conveying the antigen to APCs, extending the exposure time between antigen and immune cells, increasing antigen uptake by APCs, enhancing intracellular trafficking and controlling antigen release [89].

CONCLUSION

As an improvement over conventional vaccines, such as live, attenuated or inactivated whole organism vaccines, new generation vaccines, which are based on highly purified recombinant or synthetic antigens, stimulate effective cell-mediated and mucosal immunity. Several adjuvants are used to augment vaccine efficacy and allow administration through a non-invasive route, which requires technology for formulation development, the optimization of antigen delivery and immune potentiation. Recombinant vaccine technology is a promising technology for future vaccine development, particularly for vaccines targeting emerging and re-emerging viral infections that are life-threatening and teratogenic.

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CONFLICTS OF INTERESTS

The authors declare no conflicts of interest

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