Recombinant viruses as vectors for vaccines against human diseases – an overview of a rapidly developing field

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Abstract

Traditional vaccines for human use are based on live attenuated or avirulent organisms, killed organisms or inactivated toxoids. Advances in molecular biology and immunology within the past 30 years have led to the development of a new generation of vaccines such as the hepatitis B vaccine and polysaccharide – protein conjugate vaccine against encapsulating bacteria. An exciting prospect that promises to extend the scope of vaccines from infectious diseases to include cancer, is the use of recombinant viruses as vectors to deliver foreign proteins for immunisation. This article provides an overview, and a perspective, of the state–of- art in the field of viral vector vaccines in relation to human diseases.

Keywords: cancer, immunotherapy, infectious diseases, prophylaxis, vaccines, viral vectors, zoonotic diseases

1. Introduction

The discovery of antibiotics and vaccination are perhaps two of the most important medical advances that have led to increased human longevity in recent times. The successful global vaccination campaign against smallpox has eradicated this ancient scourge of mankind, and is a good example of vaccination saving millions of lives [1]. A related procedure, known as variolation, which is the deliberate inoculation with material from the dried scabs or pus from smallpox survivors, had been practiced for more than 1000 years as a means of inducing immunity to smallpox in China, Egypt and India. Variolation had a high incidence of failure in the sense that many recipients succumbed to a smallpox infection caused by the inoculate. However, it was Edward Jenner (1749-1823) who ushered in the modern era of vaccines when he showed that inoculation with cowpox virus (*Vaccinia*) did not cause fatalities, and successfully prevented a subsequent infection with the closely-related smallpox virus (*Variola*). Louis Pasteur (1822-1895) coined the term vaccination to honour Jenner's finding. Pasteur himself worked on the development of several vaccines, including those for anthrax, cholera and rabies. Since that time, vaccines have been used widely to prevent many other human diseases, including diphtheria, tetanus, whooping cough, polio and tuberculosis. Until recently, the so-called traditional vaccines for these bacterial and viral diseases have relied on the use of killed pathogens, avirulent or attenuated strains of pathogens, or chemically inactivated toxoids to induce protective immunity against the disease-causing organisms. The protection is mediated through vaccination inducing the formation of memory B and T lymphocytes that specifically recognise relevant antigens of the pathogen.

Advances in molecular biology over the past 30 years however have opened the possibility that pure proteins derived from pathogenic organisms, that are produced by recombinant DNA technology in bacteria and yeasts, can be used for vaccination. The first application of this approach was the development in 1986 of a vaccine against hepatitis B based on the expression of the viral surface protein in the common yeast, *Saccharomyces cervisiae* [2]. The hepatitis B virus (HBV) surface protein, which assembles as virus-like particles [2], is purified from yeast cultures, treated with formaldehyde, co-precipitated with potassium aluminum hydroxide adjuvant, and delivered intra-muscularly for vaccination. The hepatitis B vaccine has proved to be safe and highly effective, and is now routinely used in most countries. Recombinant coat proteins from human

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papilloma viruses (HPV) that similarly form virus-like particles in yeast are the basis for two recently licensed HPV vaccines for the prevention of cervical cancer and genetic warts [3].

Advances in fundamental immunology have also given rise to some novel improvements on traditional vaccines. An example is the development of vaccines based on conjugating bacterial polysaccharides to protein carriers that provide T helper cell epitopes, to stimulate IgG antibody formation and memory antibody responses against carbohydrate antigens. This approach has been effective against encapsulated bacteria such as *Haemophilus influenzae* type b (Hib), *Streptococcus pneumoniae* (pneumococci) and *Neisseria meningitidis* (meningococci) [reviewed in 4].

Even more recently, the use of recombinant non-pathogenic viruses carrying heterologous proteins derived from pathogens for vaccination has been considered to offer great potential. Such vaccines are termed viral vector vaccines and have become the subject of intense investigation in many laboratories. The perceived strength of this approach lies in the use of a live viral infection to engage pathogen-pattern recognizing receptors of the innate immune system and concurrently elicit an adaptive immune response to specific pathogen proteins. Because they mimic natural pathogen infections, viral vector vaccines do not require adjuvants, such as aluminium hydroxide, for eliciting a strong immune response. This article provides a perspective and an overview of current trends in the field of viral vector vaccines, and their potential use in preventing human diseases.

2. Types of viral vectors for vaccines

Many different viruses are being developed as vectors for vaccines. They are either harmless viruses causing self-limiting, mild infections or viruses that have been attenuated through genetic modifications to ensure that they are not virulent to the host. Adenoviruses and the modified *Vaccinia* Ankara virus (MVA) are two popular vectors for experimental vaccine development. Indeed a seminal discovery in this field was the demonstration that foreign genes could be cloned and expressed in the *Vaccinia* virus without interfering with its replication [5, 6]. Lentiviruses (*Retroviridae*), adeno-associated viruses, vesicular stomatitis virus, herpes viruses, reovirus, cytomegalovirus, and measles virus are additional examples of the many types of viruses that are in the advanced stages of laboratory evaluation or actually in clinical trials for use as viral vector vaccines. In the veterinary field, vectors such as the viruses causing fowlpox, Newcastle disease, canine distemper and Venezuelan equine encephalitis, are also being developed for use in vaccines.

Specific tropism for tissues and cell types are important properties of the viral vectors that are used for targeting specific tissue sites for generating protective immunity. For example, reovirus can target microfold or M cells in mucosal epithelia to generate mucosal immunity [7], and specific adenoviral strains also locate to the gut [8]. Lentiviral vectors have the advantage that they can infect non-dividing cells [9]. Some other properties of the viral vectors also provide important considerations for developing vaccines. These include the sites of integration into chromosomal DNA (in the case of lentiviruses and adeno-associated viruses), the robustness of the anti-viral immune response, pre-existing immunity to the virus in the human populations, and the relative ability to elicit the different arms of an effector immune response (e.g. antibodies vs helper T cells vs cytotoxic T cells). Too strong an immune response to viral vector antigens during a primary immunisation, or pre-existing immunity to the virus in a population, can diminish the efficacy of immunisation by the reducing viral replication.

3. Overcoming the problem of immunity to viral vector antigens limiting viral replication

Generation of immunity to viral antigens, that limit the use of boosting immunisations, can be overcome in a number of ways. One approach is to use bacterial plasmid DNA expressing the immunizing protein to prime the immune response before boosting with the recombinant viral vector. This approach, termed the prime-boost method, has been widely used in developing experimental malaria vaccines [10]. Another way is to use different viral vectors for the primary immunisation and subsequent boosting immunisations, and is also being trialed in experimental malaria vaccines [10]. The availability of a platform of differ-

ent adenoviruses that do not show strong immunological cross-reactions is an advantage in this regard. Adenoviral isolates from chimpanzees, which do not normally infect humans, are also available for use as viral vectors for human immunisation where pre-existing immunity to human adenoviruses in the target population is a problem.

4. Vaccination against cancer

The recombinant HBV and HPV vaccines, by preventing the corresponding viral infections, are able to reduce the incidence of liver and cervical cancer respectively. In this sense such vaccines may be considered to fall into the common category of prophylactic vaccines. However viral vector vaccines offer the potential for the therapy of a range of already established cancers i.e. they can constitute therapeutic vaccines. Advances in the field of therapeutic cancer vaccines are closely linked to the development of successful protocols for gene therapy, e.g. for severe combined immunodeficiency caused by adenosine deaminase deficiency [11]. Lentiviruses are particularly advantageous in this respect as they are able to transfect non-dividing cells, are relatively non-oncogenic and are able to stably express a large number of heterologous gene inserts [9]. Genes for T cell receptors that react with the melanoma-specific protein MART-1, and from patients who show good immune-control of melanoma, have been isolated and then transfected via lentiviruses for subsequent expression in CD4+ T cells. The transfected T cells show cytolytic and cytokine producing effector functions that are potentially useful in clinical immunotherapy of melanoma [12, 13]. The immunotherapy procedure can be enhanced by technical variations such as using the lentiviral vector to express additional immune-enhancing molecules, e.g. cytokines like interleukin-15 to expand memory T cell populations.

Advanced tumours have undergone many genetic changes that subvert the host immune system [14, 15] and therefore for immunotherapy to be most effective, it has to be performed after resection of the tumour and radio/chemo-therapy to reduce the tumour burden.

Removal of autologous antigen presenting cells (APC) from the patient, their transfection *ex vivo* with viral vectors expressing tumour specific antigens (or tumour-specific epitopes) and expansion of autologous tumour-specific T cells *ex vivo* before introduction back into the patients, are other approaches that are being investigated in the field of cancer immunotherapy. Recombinant MVA or fowlpox viruses are being used to deliver tumour-specific antigens to APC, together with molecules that increase the efficacy of antigen presentation to T cells such as CD80 and CD54. Advances in this approach to treat cancer have recently been reviewed [16]. The United States Food and Drug Administration have not yet licensed a therapeutic cancer vaccine based on viral vector delivery. However, at least two viral vector products are believed to be in phase III licensure trials for renal and cervical cancers, supported by major pharmaceutical companies.

5. Vaccination against infectious diseases

There is considerable interest in using viral vectors to vaccinate against biological warfare agents such *Bacillus anthracis* and a variety of common infectious diseases. As described below, clinical trials have been performed against human immunodeficiency virus (HIV), *Plasmodium falciparum* (Pf) malaria, *Mycobacterium tuberculosis* and Japanese encephalitis virus among other pathogens.

5.1 Vaccination against zoonotic diseases

Viral vector vaccines are already on the market for animal use in preventing zoonotic and veterinary diseases. For example, an avian influenza-fowlpox recombinant is widely used in Asia to prevent H5N1 in chickens [17] and a MVA-based influenza vaccine aiming at cross-strain protection in humans and fowl is entering clinical trials.

Inactivated whole virus vaccine is effective against foot and mouth disease (FMD) but does not permit distinction between infected and immunised animals, which is necessary in countries where the disease is not endemic but occasional imported outbreaks are possible. Similar considerations apply to bovine tuberculosis in the UK. Therefore attempts are being made to develop viral vector vaccines against these diseases that do not suffer from such a disadvantage. Viral vector vaccines based on adenovirus 5 for FMD [18] and MVA against bovine tuberculosis [19] are being developed for use in the UK and the USA.

An MVA-based recombinant vaccine against rabies, used in bait, has proved superior to attenuated rabies viral vaccines, and has been used in many countries to eliminate rabies from wild foxes that form the principal rabies reservoir among land animals [20].

5.2 HIV

Lentivirus has been used to transfect the genes for a broadly neutralising anti-HIV monoclonal antibody into haemopoietic stems cells that are then matured into B cells and plasma cells producing that antibody [21]. This is an approach that helps overcome the difficulty of generating cross-reactive and neutralizing anti-HIV antibodies through immunisation. A prime–boost approach has been used with naked plasmid DNA (engineered to express the relevant HIV proteins) followed by recombinant MVA in a number of laboratories in the USA and Europe with good safety profile, cytokine producing CD4+ and CD8+ T cell responses and antibodies that bind to the native envelope protein of the virus. Replication competent but attenuated strains of other viruses, including adenovirus are being used in the HIV field [reviewed in 22].

A major drawback in this field was the recent, highly publicized failure of Merck's Phase 2 HIV vaccine trial which was based on adenovirus strain 5 and was funded partly by the National Institutes of Health of the USA [23]. The vaccine was meant to elicit broadly-protective cell mediated immune responses in the vaccinees. Adenovirus strain 5 (Ad5) is a human virus and there is significant pre-existing immunity in human populations. Prior studies in monkey models had shown that the vaccine generated good protective immunity. However the trial went ahead despite the knowledge of pre-existing immunity in human populations because it was felt that the vaccine would overcome this and still elicit significant protective immune responses against HIV antigens. Furthermore, in sub-Saharan Africa, where an HIV vaccine is most needed, >80% of persons are estimated to have been exposed to Ad5 [23]. The trial was halted when no protection was observed and when vaccinated volunteers with high levels of Ad5 immunity were shown to have a greater propensity to become infected with HIV than unvaccinated controls. A tendency for the vaccine to protect was however seen in those vaccinated persons with low levels of immunity to adenovirus. The cause for possibly enhanced HIV infectivity in persons with higher levels of pre-existing antibodies to Ad5 is not clear but recent *in vitro* studies with dendritic cell and T cell co-cultures show that Ad5-antibody immune complexes activate the dendritic cells and increase HIV infection of the cells [24].

Recently it was demonstrated that colorectal immunisation with a recombinant adenovirus carrying herpes simplex virus-2 (HSV-2) glycoprotein B was able to protect mice against a lethal rectal or vaginal challenge with HSV-2 [25]. This finding suggests that a similar approach may be successful against HIV.

5.3 Malaria

The most advanced vaccine against malaria, that is currently undergoing Phase 3 trials, is based on the recombinant *Plasmodium falciparum* circumsporozoite protein produced in yeast in fusion with the hepatitis B virus surface antigen as virus like particles. This vaccine is termed RTS, S. When this vaccine is administered with the best available adjuvant licensed for human use, protection against severe falciparum malaria approaches about 50% [26]. There are also several other malaria vaccines under pre-clinical development [reviewed recently in 27]. My own laboratory has investigated the use of the food grade bacteria, *Lactococcus lactis*, *Lactobacillus reuteri* and *Lb. salivarius*, as vectors for experimental mucosal immunisation of laboratory animals using a *P. falciparum* merozoite surface antigen with promising results [28, 29]. We have also examined the use of a recombinant plant virus, cowpea mosaic virus, to immunise animals with peptide epitopes based on a different *P. falciparum* merozoite surface antigen, but the results showed that the immune response to the virus-specific antigens was too highly dominant for the method to be useful for human vaccination against malaria [30].

However viral vector vaccines based on MVA and adenovirus either used alone or in combined prime-boost strategies with plasmid DNA, are also in clinical trials. These vaccines are based on proteins located on

the surfaces of sporozoites and merozoites, and are designed to elicit effector T cells as well as antibodies [10, 31].

5.4 Tuberculosis

While BCG protects against disseminated tuberculosis (TB) in young children living in non-endemic countries, its efficacy in TB-endemic areas and in adults is in doubt. Based on successful tests in mice and guinea pigs as well as cattle, and encouraging early clinical trials [19], clinical Phase 3 trials using MVA or adenovirus expressing the protective bacterial antigen mycolyl transferase, to enhance BCG -induced immunity in Africa, are being planned.

5.5 Japanese encephalitis

The live attenuated yellow fever virus (YFV) vaccine has a long record of efficacy and safe use in human populations with several hundred millions already vaccinated with it [32]. Efforts are therefore underway to insert genes from other flaviviruses into the YFV to generate chimaeric viruses that can be used for vaccination. An effective vaccine against Japanese encephalitis (JE) using this approach is of particular value due to shortcomings of the existing inactivated and attenuated JE virus vaccines. Based on encouraging pre-clinical trial results [33], it is expected that a chimaeric YF-JE vaccine will be licensed soon for human use.

6. Licensing and safety issues with viral vector vaccines

The production and use of viral vector vaccines entail manufacturing challenges, especially to ensure low cost supply for less affluent countries and livestock markets, safety issues relating to the use of live viruses on a large scale, and environmental issues related to their release or shedding in urine and faeces. The genetic stability of attenuated viruses used for vaccination has to be carefully monitored. The production of recombinant, replication defective, viruses for immunisation involves packaging reactions where helper plasmids coding for critical viral proteins that are missing from the attenuated viral vector are utilised. Recombination between these to generate replication competent virus is possible and requires appropriate safeguards. There is also the possibility that the attenuated or replication defective viral vector can recombine with naturally circulating virus strains to yield virulent viruses. Insertion of the virus sequence into host DNA, however rare, can cause insertional mutagenesis leading to cancer. These potential hazards depend on the nature of the vector virus and can be mitigated in a number of ways.

Additionally, the use of the same viral vector, say adenovirus 5, for delivering vaccines against TB, HIV and malaria in countries where all three diseases are co-endemic, will raise issues regarding anti-vector immunity interfering with vaccine efficacy. The use of different viral vectors or vector strains is expected to avoid this problem. Some of the safety issues with viral vector vaccines have been highlighted recently by the preliminary stoppage of a Phase IIb efficacy trial of Merck's adenovirus 5-based vaccine for HIV [23]. The failure of this vaccine is attributed to the high levels of natural immunity in the vaccinees to the adenovirus 5 vector. This drawback may be overcome by using an adenovirus strain for which there is little human immunity, e.g. a chimpanzee adenovirus.

7. Conclusions

The development of effective and safe vaccines for human use is always a lengthy process, inevitably accompanied by pitfalls. With further advances in the relevant molecular biosciences and technology, it can however be safely predicted that viral vector vaccines will eventually become useful components of our prophylactic armoury against a variety of serious infectious diseases of humans and animals. Virally delivered therapeutic vaccines against cancer are also likely to contribute in a major way to the successful remediation of many forms of human cancers where the traditional treatment methods of surgical excision, chemotherapy and radiotherapy are only partially successful.

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