Chapter 1

Recent developments in vaccines strategies against human viral pathogens

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1 Introduction

Emerging diseases affecting livestock and humans represent an important threat to public health and the world economy. Various parameters such as increasing urbanization, international trade and commerce, or climate change favor the likelihood that the threat of emerging pathogens will continue, if not worsen, in the future. Immunization is arguably the most appropriate way of preventing these emerging infectious diseases. Vaccination is one of the most efficient tools against infectious diseases which has led to a significant reduction in mortality and morbidity. On a global level, it is one of the few cost-effective medical measures that result in universal benefit and perhaps one of the outstanding achievements of medical intervention. Vaccine-induced immunity that is established in advance of virus infection relies primarily on adaptive immune responses for protective efficacy. Critically, vaccination depends on the properties of antigen recognition, activation, expansion, memory, trafficking, and the multitude of specialist functions of lymphocytes. The extent to which vaccine-induced immunity is successful also determine the spread and maintenance of a viral pathogen within a population. Viral vaccines have had profound and enduring consequences for human and animal health; the worldwide eradication of smallpox in 1980 (Henderson, 1980) and rinderpest is a testament to their outstanding contribution to modern society.

Nevertheless, infectious diseases still pose one of the greatest threats to public health and the past three decades have brought a constant barrage of new human pathogens. More than 70% of these infections are zoonotic (Jones et al., 2008; Woolhouse et al., 2005), entering either directly from wildlife reservoirs or indirectly via an intermediate domestic animal host (Henderson, 1980; Field, 2009). HIV, avian influenza, Hendra (HeV) and Nipah (NiV) viruses, severe acute respiratory syndrome (SARS) and Middle East respiratory syndrome coronavirus (MERS-CoV), Ebola and Marburg filoviruses, Lassa virus (LASV), Rift Valley fever virus (RVFV), and Crimean-Congo hemorrhagic fever (CCHF) virus are all examples of zoonoses currently emerging from wildlife. All these emerging zoonoses present a serious and increasing threat to health, biosecurity, and economies worldwide. The mechanisms underlying disease transmission from animals to humans are becoming better understood (Kreuder Johnson et al., 2015) with the emergence of pathogens from wildlife (which represents the greatest threat to global health) occurring in a nonuniform pattern, being localized to distinct geographic "hotspots" in Africa, Asia, and South America, and with each high-threat pathogen being weighted toward a key wildlife species [e.g., bats, rodents, or nonhuman primates (NHPs)]. It is clear that such diseases will continue to place a substantial burden on global health, especially in dense human populations where the pressures on environmental and economic resources are greatest. More than one billion cases of human zoonotic disease are estimated to occur annually, and emerging zoonoses result in enormous economic losses (Karesh et al., 2012). Increased urbanization, international travel, commerce, and climate change increase the likelihood that emerging zoonosis will continue, if not worsen, in the future.

In 2014, the unpredicted size, speed, and reach of the Ebola virus outbreak in West Africa (WHO Ebola Response Team, 2016) acted as a wake-up call for researchers, pharmaceutical communities, and governments, emphasizing the importance of investment into the study of emerging pathogens. Spurred on by this development and at the request of its 194 Member States in May 2015, the World Health Organization (WHO) convened a broad coalition of experts to develop a research and development (R&D) Blueprint for Action to Prevent Epidemics. Focusing on severe emerging diseases with the potential to generate public health emergencies, and for which no, or insufficient, preventive and curative solutions exist, the R&D Blueprint specifies R&D needs, including vaccine research. Through international governance, the program aims to define R&D roadmaps for prioritized pathogens and to catalyze funding strategies (WHO, 2020).

Development of a vaccine against an emerging virus faces several uphill tasks. Conventional vaccination strategies, based on an inactivated virus or live attenuated strains, have been instrumental in the control and even eradication of some important animal and human viral diseases. But this strategy falls short in many other cases where they fail to deliver the required levels of immunogenicity, safety, cross-protection across the viral antigenic variability, or even exacerbate disease (García-Sastrea and Mena, 2013). This implicates the need to develop novel vaccine strategies.

Vaccines are developed by employing different strategies. They may contain live viruses that have been attenuated (weakened or altered so as not to cause illness), inactivated or killed viruses, inactivated toxins (for bacterial diseases where toxins generated by the bacteria, and not the bacteria themselves, cause illness), or merely segments of the pathogen (this includes both subunit and conjugate vaccines). Critically, vaccination depends on the properties of antigen recognition, activation, expansion, memory, trafficking, and the multitude of specialist functions of lymphocytes. Design of a viral antigen, a delivery system that maximizes antigen presentation and induces broad-spectrum immune responses, is key to the development of any successful and effective vaccine. The recent advancements in the fields of virology, immunology, molecular biology, and vector delivery strategies yielded a tremendous understanding of cellular mechanisms of adaptive immune responses, paving the way for the development of novel vaccines against emerging viral diseases. The vaccines delivered using inactivated, live-attenuated, and virus-like particles (VLPs) are the most successful strategies which are still used in large. However, there has been some current novel vaccine approaches for safe and effective vaccines encompassing recombinant virus technology, nucleic acid vaccines, and self-disseminating vaccine approaches (Graham, 2013). Various strategies for the development of viral vaccines with some of the examples and their advantages and disadvantages are present in Table 1. Here we discuss some of the recent developments in vaccine strategies.

2 Virus-like particles

Virus-like particle (VLP) technology is a very powerful method for developing vaccines (Noad and Roy, 2003; Chackerian, 2007; Roldao et al., 2010). In simple terms, VLPs are molecules that closely resemble viruses but are noninfectious because they contain no viral genetic material. In the virion, structural proteins are usually arranged in tight and well-ordered conformation, which is believed to be recognized as a pathogen-associated molecular pattern (PAMP). Therefore, one way to increase the immunogenicity of viral antigens is to deliver them in

TABLE 1 Novel vaccine strategies against emerging viral diseases.

Vaccine platform	Example	Advantages	Disadvantages
Viral vector	DENV, YFV, RVFV, VSV, EBOV	Elicit humoral and T cell responses. High level of antigen expression Several vector platforms with different profiles available.	Preexisting immunity to the vector can decrease efficacy.
Virus like particles	CHIKV, IAV, HBV	Multimeric presentation of the antigen in native conformation. Safe, no viral replication.	Production yields, cost and purification can be limiting
Naked nucleic acid	Dengue Virus	Safe: no viral replication. Elicit humoral and T cell responses. Replicons have increased immunogenicity	Poor immunogenicity (but can be enhanced by adjuvants and heterologous prime-boost strategies).
Recombinant and synthetic peptides	HeV IAV	Safe: no viral replication. Can direct the response to conserved epitopes.	Poor immunogenicity. Might require the use of potent adjuvants or boosts.
Recombinant bacteria	SARS-CoV	Adjuvant effect of the vector, Low cost, mass production	Limited experimental information, no clinical trials

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multimeric conformation and as VLPs (Roldao et al., 2010; García-Sastrea and Mena, 2013). VLPs based on both enveloped and non-enveloped viruses can be used to immunize against the homologous virus or engineered to incorporate epitopes from a different pathogen. Interest in the use of VLPs as vaccine candidates stems from their ability to present ordered and highly antigenic structures to the immune system. At the same time, they lack a viral genome, potentially yielding safer vaccines, as there is no viral sequence that can revert to virulence. Furthermore, through genetic fusion or chemical conjugation, VLPs have become attractive carrier proteins of foreign antigens, since they can efficiently display them within a host immune system (Plummer and Manchester, 2011; Tissot et al., 2010; Brune et al., 2016). The optimal structural characteristics of VLPs offer a platform to achieve the desired immunological effects and provides an overview of the agonists of
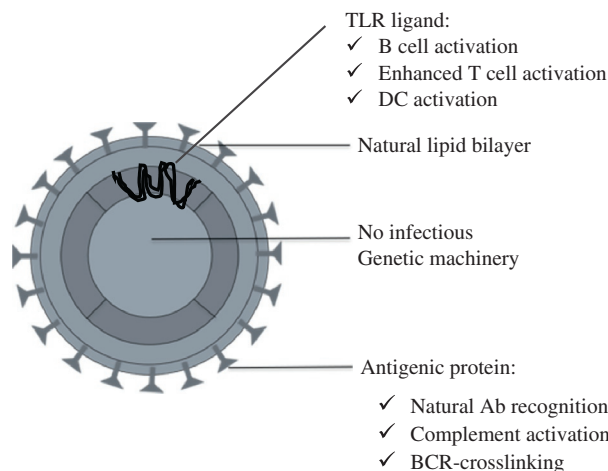
TLR ligand:
✓ B cell activation
✓ Enhanced T cell activation
✓ DC activation

Natural lipid bilayer

No infectious
Genetic machinery

Antigenic protein:

✓ Natural Ab recognition
✓ Complement activation
✓ BCR-crosslinking

FIG. 1 Different modes of immune response induction by VLPs or recombinant viral proteins.

the toll-like receptors family (TLR) members, that can be cloned into the construct for the expression of VLPs, as a metod to enhance the immune responses (Fig. 1) (Mohsen et al., 2017). They induce strong B cell responses in the absence of adjuvants by efficiently cross-linking specific receptors on B cells, and can also trigger T cell-mediated responses. An important advantage of VLP-based vaccine platforms is that VLPs can present antigens in a dense and repetitive manner, thus effectively enabling the cross-linking of B cell receptors (BCRs) or in other terms drive rapid differentiation of memory B cells, producing increased levels of antibodies (Zabel et al., 2014). Multivalent antigens bound to BCRs show more efficient processing and presentation than monovalent antigens. Cross-linking of BCRs by multivalent antigens leads to a conformational change in BCRs and induces signaling-active BCR microcluster formation, leading to robust B cell activation and signaling (Casten and Pierce, 1988). Therefore, VLP-based approaches offer great potential for developing effective vaccines against many pathogens, including antigens known to be weakly immunogenic. The basis of ordered viral self-assembly from protein subunits that is noted in many different virus families provides the foundation for work with VLPs, with more than 30 different viruses that infect humans or other animals being identified as able to produce VLPs. They are structurally diverse, having single or multiple capsid proteins, or a lipid envelope, although not all viruses are suitable VLP candidates. Those which are can elicit a protective response without requiring multiple booster shots, thus significantly reducing the vaccine costs. To date, VLP-based vaccines for human papillomavirus (HPV), hepatitis B virus (HBV), and hepatitis E virus (HEV) have been licensed and are commercially available worldwide (Jain et al., 2015). Several VLP-based vaccine candidates for human diseases are under clinical development, including those directed against Norwalk virus,

Zika, influenza A, Ebola and Marburg viruses, and hepatitis C virus. VLP vaccines combine many of the immunogenic advantages of whole-virus vaccines with the safety advantages of recombinant subunit vaccines.

3 Recombinant proteins and synthetic peptides

Recombinant proteins and synthetic peptides can be termed as a safe vaccine strategy to induce immune responses via the delivery of a viral antigen produced by recombinant methods or chemical synthesis. Synthetic peptide-based epitope vaccines make use of short antigen-derived peptide fragments that can be presented either to T cells or B cells (Purcell et al., 2007). The choice of an epitope is a crucial step in the design of a peptide-based vaccine. At first, appropriate epitopes on the protein of interest need to be identified. These epitopes should be able to induce strong, long-lasting humoral and/or cellular immunity against the desired pathogen. However, epitopes chosen for peptide vaccine design are not always the immune-dominant epitopes against which humans predominantly induce immune responses. Recombinant protein vaccines offer several advantages such as safety, production does not require the pathogen, avoiding the risk of accidental escape and the hurdles of high biosafety and containment requirements. The design of vaccine candidates is possible even when there is limited information about the pathogen. Also, subunit vaccines can be used to overcome the natural immunodominance of highly variable epitopes and direct the immune responses against conserved and broadly protective epitopes (García-Sastrea and Mena, 2013).

The main disadvantage of subunit/synthetic vaccines is that they are usually poor immunogens as they failed to be recognized as PAMPs and activate innate immune responses, which are required for the full development of acquired immunity. To overcome this problem, they are usually presented in an immunogenic confirmation and/or accompanied by potent adjuvants. One such example of this approach is a vaccine candidate based on the envelope glycoprotein of the BSL-4 pathogen Hendra Virus (HeV) (family Paramyxoviridae, genus Henipavirus) that has been shown to induce complete protection in a ferret model (Pallister et al., 2011).

As T-helper cells play a crucial role to link innate and adaptive immunity, T-helper epitopes are exploited as the crucial components of peptide-based vaccine. While immune responses without the presence of T-helper are possible, they are often weaker, uneven in heterogeneous population and memory responses are impaired. T-helper epitopes can be both disease-specific (derived from the protein of the targeted pathogen) and universal, such as an artificial pan-DR helper T-lymphocyte epitope

(PADRE). This synthetic peptide was designed to bind most of the human HLA-DR receptors, providing "universal" immune stimulation in a heterogeneous population. Thus, incorporation of universal T-helper epitopes in peptide-based vaccine has become a very successful approach since their discovery in the 1990s (Alexander et al., 1994; Overholser et al., 2015).

4 Recombinant bacteria as vaccine vectors

In addition to being extensively used to produce recombinant subunit vaccines, bacteria can also serve as vectors for the in vivo delivery of antigens or DNA. The advantages of this platform is the low cost and easy to scale-up production, the availability of well-characterized attenuated bacterial strains, the activation of the innate immunity by the vector, and the efficient delivery to antigen-presenting cells. Several genera are being explored as vaccine vectors, including Listeria, Salmonella, Lactococcus, and Bordetella. Recombinant bacteria can be used as live attenuated vaccines, inactivated, or even as cytoplasm-depleted bacterial ghosts. Recombinant Lactococcus lactic expressing the N protein of SARS coronavirus has been shown to induce antibodies in mice (Pei et al., 2005). The recombinant Bordetella pertussis expressing the influenza virus eM2 induces high titers of specific antibodies in mice but failed to elicit protection in mice (Li et al., 2011). *Listeria monocytogenes* hemolysin transport system secreting the nucleoproteins of either influenza virus or lymphocytic choriomeningitis virus (LCMV) was shown to result in strong CTL responses (Ikonomidis et al., 1994; Goossens et al., 1995).

5 Nucleic acid vaccines

DNA vaccines are third-generation vaccines. They contain DNA that codes for specific proteins (antigens) from a pathogen. DNA vaccines have emerged as a safer alternative to standard live and inactivated vaccines for treating human and animal viral diseases. They exhibit several advantages over traditional strategies in terms of safety, stability, ease of manufacturing, and immunogenicity. They offer greater advantages for vaccination against emerging and reemerging viruses, in that plasmids expressing a viral antigen can be easily and rapidly produced, which can be expressed in in vivo and induce both humoral and cell-mediated immune responses. Large quantities of DNA can be produced in a short time at a reduced cost, and DNA preparations are more stable than other types of vaccines, which are desirable properties for a vaccine, especially when they are supposed to be transported to remote areas. DNA vaccines are considered very safe, suitable for DIVA applications, and not affected by anti-vector immunity. However, the biggest limitation in the development of DNA vaccines is their intrinsic low immunogenicity. Work to improve this has focused on optimizing delivery approaches with the use of gene guns, or electroporation; targeting immune effector cells; and the use of potent adjuvants. DNA vaccines are also frequently used in combination with other vaccines platforms in heterologous prime-boost strategies for efficient immune responses.

A DNA vaccine is currently licensed to immunize horses against WNV and has undergone phase I clinical trials even in humans (Ledgerwood et al., 2011). DNA vaccines have been evaluated as candidates against many emerging viruses, including EBOV, RVFV, dengue virus, and CHIKV (Martin et al., 2006; Boshra et al., 2011; Porter et al., 2012; Mallilankaraman et al., 2011).

Replicon vaccines are based on defective RNA genomes that are able to undergo replication and express encoded proteins but cannot produce infectious viral particles. Viral RNA replication is a strong inducer of the innate immunity and, therefore, has superior immunogenicity than the equivalent DNA vaccines (Ulmer et al., 2012).

6 Viral vector technology

The first published description of using a recombinant virus to deliver antigens from another infectious agent was the recombinant vaccinia virus engineered to express hepatitis B surface antigen in animal cells, which upon immunization in chimpanzees induced protective immune response against HBV (Moss et al., 1984). Advances in recombinant DNA technology, molecular biology, and virus reverse genetics have provided key insights into the replication and pathogenesis of a wide range of viruses lead and still leading to the development of various recombinant viral vectors for vaccine and immunotherapeutic applications. This advancement has facilitated the development of various virus vectors for protein expression and vaccination. To date, several virus families have been exploited as vectors, including many for vaccination (Hewson, 2000; Small and Ertl, 2011; Rollier et al., 2011; Ljungberg and Liljeström, 2015). One of the biggest advantages of viral vector vaccines is that the choice antigen is expressed in the context of an active heterologous viral infection, which stimulates the full activation of innate immune responses required for the development of adaptive humoral and T cell-mediated immunity (Liu, 2010). Another vital aspect of a virus-vector vaccine for emerging viruses is that the characteristics, and intensity of the immune responses, as well as safety considerations and manufacturing technology, are predominantly defined by the vector and not the pathogen. Thus developing and testing a vaccine against a newly emerged virus can be significantly shortened by this strategy (Afrough et al., 2019).

Viral vector vaccines combine many of the positive qualities of DNA vaccines with those of live attenuated

vaccines. Like DNA vaccines, viral vector vaccines carry DNA into a host cell for the production of antigenic proteins that can be tailored to stimulate a range of immune responses, including antibody, T-helper cell (CD4+ T cell), and cytotoxic T-lymphocyte (CTL, CD8+ T cell) mediated immunity. Unlike DNA vaccines, these vector vaccines also have the potential to actively invade host cells and replicate, much like a live attenuated vaccine, further activating the immune responses like an adjuvant (Draper and Heeney, 2010).

Though viral vector vaccines are superior to produce stronger immune responses than DNA vaccines, for some diseases viral vectors are being used in combination with other vaccine technologies in a strategy called heterologous prime-boost. In this system, one vaccine is given as a priming step, followed by vaccination using an alternative vaccine as a booster. The heterologous prime-boost strategy aims to bolster a stronger overall immune response. Viral vector vaccines are being pursued as both prime and boost vaccines as part of this strategy.

There are no viral vector vaccines on the market for use in humans, although many are under production and early phase trials. There are about 12 viral vector vaccines currently in use for veterinary diseases. The approved vaccines include adenovirus, fowlpox virus, attenuated yellow fever (YFV-17D), and vaccinia virus vectors, all of which are relevant as potential human viral vector vaccines (Draper and Heeney, 2010).

6.1 Adenovirus vectors

Human Ad serotype 5 (Ad5) has been widely investigated as a gene delivery vector among all the adenoviruses identified as it can be easily produced in high titers. Recombinant Ad vectors are highly exploited as vaccine vectors because of their high transduction efficiency, high level of transgene expression, and a broad range of viral tropism. Adenoviruses can also infect both dividing and nondividing cells. Most Ad vectors are replication-defective due to the deletion of the E1A and E1B viral region. Often, the E3 genes are also deleted to provide space for the transgene insertion. Adenoviral vectors can induce innate immune responses via Toll-like receptor-dependent and Toll-like receptor-independent pathways. As Ads can infect dendritic cells (DCs), consequential upregulation of co-stimulatory molecules accompanied by increased cytokine and chemokine production by the infected DCs can contribute to effective antigen presentation to the immune cells (Banchereau and Steinman, 1998).

As most people have been exposed to any of the Adenovirus serotype, the presence of preexisting immunity is a disadvantage of these vectors. Ad contains three main structural proteins, hexon, penton, and fiber, and these proteins

are the major targets of the humoral and cellular immune responses (Sumida et al., 2005). Antibodies against the hypervariable regions (HVRs) of the hexon protein dominate the neutralizing responses and modification of these HVRs and the fiber knob domain has been investigated as the best way to evade preexisting immunity against these vectors (Roberts et al., 2006; Ura et al., 2009).

The use of Ad vectors has been explored in the area of HIV vaccine development. The effectiveness of the recombinant Ad5-based vaccine, expressing HIV-1 gag, pol, and nef genes as target antigens, was evaluated in a nonhuman primate model (Gabitzsch et al., 2009; Barouch, 2010). The recombinant Ad vector vaccines for other viruses under different phases of development and clinical trials (being tested for animal and human use) are presented in Table 2.

6.2 Poxvirus vectors

Vaccinia virus, a member of the poxvirus family, is a large, complex enveloped virus. The virus was traditionally used for the production of smallpox vaccine (smallpox is declared to be eradicated long back), and its efficacy and safety

TABLE 2 List of Adenovirus vector-based vaccine candidates.

Viruses	Ad vectors	Antigens/ transgene expressed	References
HIV/SIV	AdHu5, AdHu26, AdHu35	Gag, Pol, Env, Nef, GRIN	Buchbinder et al. (2008), Churchyard et al. (2011), Frahm et al. (2012) Janes et al. (2012)
Influenza virus	AdHu4, AdHu5	HA	Kampen et al. (2005), Gurwith et al. (2013)
Ebola virus	Chimpanzee Ad3, AdHu5, AdHu26, AdHu35	GP	Ledgerwood et al. (2010), Sullivan et al. (2011)
Rabies virus	AdHu5, Canine Ad type 2, AdC68	GP	Prevec et al. (1990), Bouet-Cararo et al. (2011)
Dengue virus	AdHu5	E, prM	Raviprakash et al. (2008), Khanam et al. (2009)

have been well demonstrated. During vaccine development, highly attenuated vaccinia virus strains have been generated, including replication-competent (LC16m8) and replication-deficient (NYVAC, ALVAC, TROVAC, and MVA) strain and these strains are most frequently used for vaccinia virus vector production. Some attenuated poxvirus vectors, such as ALVAC, modified vaccinia virus Ankara (MVA), NYVAC, and Fowlpox are the most promising vectors and have already entered into a large number of clinical trials with some of them rapidly moving forward into more advanced prophylactic and therapeutic clinical trials as vaccines against many diseases.

Modified vaccinia Ankara (MVA) is a highly attenuated strain derived from the vaccinia strain Ankara. About 15% of the vaccinia genome and the ability to replicate in mammalian cells was lost in the MVA and has been safely administered to over 120,000 individuals as a smallpox vaccine (Stickl et al., 1974).

The first vaccinia virus-based gene expression vector was described in 1982 (Mackett et al., 1982). Since then, many studies have shown that vaccinia virus vector-based vaccines can induce a robust immune response against various foreign antigens because of high transgene expression (Gomez et al., 2011a). The vectors also activate a strong innate immune response mediated by TLRs and the inflammasome, resulting in an adjuvant effect (Zhu et al., 2007). Currently, many clinical trials are evaluating the use of vaccinia virus vectors in viral diseases such as HIV-1, hepatitis, rabies, HCMV, HBV, Lassa, influenza, Equine herpesvirus, and other viral diseases (Gomez et al., 2011b; Cavenaugh et al., 2011; Berthoud et al., 2011; Sánchez-Sampedro et al., 2015). Most of the vaccines are intended to induce a robust CTL response against foreign antigen. In clinical trials, although vaccinia virus vectors were well tolerated, severe adverse events were also reported in some studies when the MVA vector was administered in a dosage-dependent manner (Gilbert, 2013). Even though none of the poxvirus vectors have been approved for human use as a recombinant viral vaccine, the promising results from a large database of preclinical and clinical trials indicate a not-too-distant application of these type of vaccine for humans. A phase-III ALVAC-based HIV-1 vaccine demonstrated a modest protective effect and this was the first trail to provide evidence for the efficacy of an HIV-1 vaccine in a large-scale study (Rerks-Ngarm et al., 2009) and in the recent outbreak of Ebola after highly encouraging efficacy results were observed in nonhuman primates with the prime/boost combination of adenovirus and MVA vectors expressing Ebola GP protein, a protocol that might be implemented as part of phase-I/II clinical trials have been initiated at various sites in Africa. There is also abundant preclinical information on the proven efficacy of these vectors in other model diseases.

6.3 Adeno-associated virus (AAV) vectors

Due to its unique biology, simple structure, and no known disease association, AAV has become the preferred carrier for most gene therapy applications and vaccine vectors. A safe clinical profile, availability of viral serotypes targeting different tissue tropisms, and potential long-term gene expression are main advantages of rAAV as viral vectors. Gene therapy using AAV has been demonstrated to be safe and well tolerated in almost all clinical settings, which reveals that this vector has important application value in many respects. Another remarkable feature of AAV vector vaccines is their ability to induce strong and long-lasting antibody responses. Several studies have documented the induction of strong humoral responses that could last for many months (Manning et al., 1997; Ploquin et al., 2013). AAV is a very small (20–26nm) icosahedral and non-enveloped virus belonging to the *Parvoviridae* family. The AAV particles contain a single-stranded DNA genome of the size of about 4.7 kb, which includes two major open reading frames, Rep and Cap.

The propagation of AAV in tissue culture requires the help of another virus in which adenovirus (AdV) and herpes virus (HSV) are traditionally used as AAV helper viruses. The AAV vector is a recombinant variant of the wild-type AAV virus in which the native coding region and the non-coding region have been replaced by the gene of interest. The vector genetic construct retains the lateral ITR, which is the only cis-acting element required for AAV DNA replication and encapsidation (Fig. 2). Through these modifications, recombinant adeno-associated viruses (rAAV) becomes a replication-defective entity that can only infect cells and deliver DNA to the nucleus. The important aspects of rAAV vector production include host cell lines that produce rAAV as well as the design of AAV genes, promoters, and regulatory elements. Adjusting the expression of these elements not only contributes to increase productivity but also improves process robustness and product quality. Currently, four commonly used rAAV expression systems include adenovirus, herpesvirus, baculovirus complementary systems, and yeast expression systems (Aponte-Ubillus et al., 2018).

AAV8 and AAVrh32.33 vector-based genetic vaccines encoding truncated dengue virus envelope proteins have been reported to elicit a long-lasting humoral response in mice (Li et al., 2012). rAAV2/8 and rAAV2/rh32.33 encoding HCV E2 glycoprotein was also shown to elicit humoral immune responses in mice (Zhu et al., 2019). Various active immunization studies employing rAAV vectors against several viruses such as HIV, SIV, HPV, HSV-1, SARS-CoV, and influenza viruses highlight the advantages of the advantages of rAAV-based vaccines (Nieto and Salvetti, 2014).
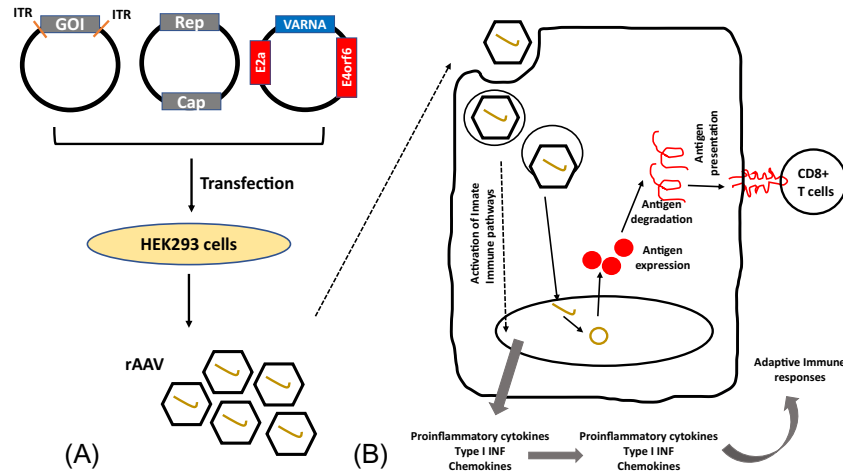
FIG. 2 (A) Production of recombinant AAV: the vector AAV DNA containing the gene of interest (GOI), the Rep & Cap constructs and the helper constructs for adenovirus genes. All the constructs were transfected into the HEK293 cells for production of rAAV. (B) Cartoon showing the mechanism of immune response induction by rAAV vector based vaccines.

6.4 Other viral vectors

Apart from these virus vectors several retroviruses, lentivirus, cytomegalovirus, and Sendai virus-based vectors were also developed for their use as vector vaccines. Among the retroviral vectors, Moloney murine leukemia virus (MoMLV) has been widely studied for vector vaccines. Lentiviruses, a subclass of retroviruses, can infect both non-dividing and dividing cells, whereas retroviruses infect only dividing cells. Thus, lentiviruses generally exhibit broader tropism than retroviruses. The advantages of the lentiviral vector are quite like those of retroviral vectors. Although lentiviruses can potentially trigger tumorigenesis, the risk is lower than that of retroviral vectors, as the integration sites of lentiviruses are away from the sites harboring cellular promoters.

Cytomegalovirus (CMV) is a member of the herpesviruses. CMV often goes unnoticed because its pathogenicity is very mild in people who are immunologically healthy. But pregnant women and immunocompromised individuals are highly vulnerable to its pathogenesis. A rhesus CMV (RhCMV) vector-based vaccine shown to protect against SIV infection and also eliminated SIV (Hansen et al., 2011). Interestingly, the RhCMV-based vaccine was also shown to elicit unique MHC class II-restricted CTL responses recognizing a broad range of antigen epitopes. Such features have not been observed with MVA- or Ad5-based vaccines (Hansen et al., 2013).

Sendai virus (SeV) is an enveloped, single-stranded RNA virus of the family Paramyxovirus. The SeV vector exhibits highly efficient gene transfer, transduces both dividing and nondividing cells and human airway epithelial cells, and is often administered through mucosal (oral and nasal) route. Intranasal administration can potentially reduce the influence of a preexisting immunity to SeV, as compared to intramuscular administration. The SeV vector has been used for gene therapy and also in a vaccine in human trials (Slobod et al., 2004; Nakanishi and Otsu, 2012).

7 Future perspectives and conclusion

Emerging and reemerging viral diseases, especially zoonotic diseases, can present many challenges and great risks for global health and economy. The 2014 reemergence of the Ebola virus in African countries have given clear warnings about the danger of these devastating pathogens on global health and raised an alarm about the preparedness in facing such a global health issue. Innovating and adopting novel vaccination strategies is the only way forward to specifically address these challenges. Many novel vaccination strategies that have been developed during recent years have the potential to specifically address the growing threat of new and emerging viral diseases. We also inevitably will be faced with emerging viral diseases for which vaccines would benefit public health. Addressing these challenges will require new paradigms for discovery, development, manufacturing, and distribution. Future research will be needed for the continuous improvement of the safety and immunological characteristics of various vaccination strategies.

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