Review on Adeno Virus; As a Vaccine Vehicle

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Abstract

Adenoviruses have moved to the forefront of vaccinology and are showing substantial promise as vehicles for antigen delivery for a number of vaccines currently being developed. Most studies to date have focused on human serotype adenoviruses, particularly human adenovirus type 5. Human serotype adenovirus vaccine vectors are particularly useful for development of veterinary vaccines as neutralizing antibodies to the vector will not usually be present in the vaccinates. Most vectors currently used as vaccine carriers are deleted in E1 gene. The original E1 deleted adenoviral vectors were constructed by homologous recombination. Replication incompetent vectors contain an antigen expression cassette substituted for the deleted E1A– E1B region. These replication incompitant adenoviruses can not replicate because of the deletion of the essential viral E1 gene region containing two genes. Replication competent adenoviral vectors encode all of the remaining adenoviral antigens in addition to the transgene product, i.e., the vaccine antigen. The potential for adenoviruses to elicit powerful B cell and T cell responses in the mammalian host are the main reason for the use of these vectors in vaccine development. For effective veterinary use, extensive research on adenoviral vaccine vectors should be undertaken.

Keywords: Adenovirus; Deletion; Recombination; Tropism; Vaccine

Introduction

Microbial vectors have been developed for several reasons: to ensure efficient delivery to somatic cells, to increase the antigen produced, and to increase their potency by the provision of adjuvant or the activation of innate immune responses. Viruses have evolved highly efficient structures and mechanisms for infecting cells and utilizing the cellular machinery for production of virally encoded proteins. Thus, viral vectors are natural preferred vehicles for heterologous gene delivery for immune responses and have been extensively studied and developed as such [1].

Most viral vectors are developed using viruses that are associated with mild or no disease or using viruses that are pathogenic but attenuated by deletion of virulence genes. Replication competent virus vectors, which can produce progeny virus, as well as replication-defective virus vectors, which do not produce progeny

virus, have been developed and evaluated as vaccine delivery vehicles [2].

A number of viral vectors have been developed or are in the process of being developed, improved and evaluated. These include DNA viruses such as Adenoviruses, Herpes viruses and Pox viruses that have been used in a wide range of applications [3,4] where as replication deficient human Adenovirus vectors have been used very successfully in the development of foot and mouth disease virus vaccines [5]. The major advantage of live vectors is that they produce the antigen in its native conformation, which is important for generating neutralizing antibodies and can facilitate antigen entry into the MHC class I processing pathway for the induction of CD8+CT [6].

Adeno viruses have shown tremendous promise as delivery vehicles for recombinant vaccination and gene

therapy. Adeno virus vectors are suitable candidates for gene delivery applications. Adenoviruses are ubiquitous pathogens associated with mild or no diseases in immune competent animals such as birds, mammals, reptiles and amphibians. The ability to infect a wide range of actively dividing and non dividing mammalian cells and to induce a high level of transgene expression made Adenovirus to develop high levels of antigen specific humoral and cell mediated immune responses in response to vector delivery either via a systemic or mucosal route. The adenoviral genome is well characterized, comparatively easy to manipulate and minimum risk of integration into the host genome. They can be applied systemically as well as through mucosal surfaces and their relative thermo stability facilitates their clinical use [7].

Therefore, the objectives of this seminar paper are:

- To outline the basic principles of adenovirus vector construction.
- To provide insight on the application and challenges of adenovirus as a vaccine vector.

Adeno Virus Structure and Replication

Virion Property

Adenoviruses were first isolated from cultures of human adenoid tissue (tissue located at the back of the nose, above the tonsils) and were discovered in 1953. Adenoviruses are medium sized (90–100 nm), non enveloped, icosahedrally symmetric double stranded DNA viruses. The genome of adenovirus is approximately 34 - 43kb and to conserve space, it encodes polypeptides from both DNA strands and uses alternative splicing and different polyadenylation sites [8].

The adenovirus capsid is composed primarily of 240 homotrimeric hexon capsomers with twelve Penton capsomers located at each of the twelve fivefold axes of symmetry. The capsid of the virus contains three major proteins: Hexon, Fibre and Penton Base proteins. The Hexon protein provides structural support, but differs in size and immunological properties between serotypes. The fiber protein of adenovirus exhibit high homology among serotypes and occurs as a trimer on the surface of the capsid. The fibre proteins are anchored to the capsid by the penton base proteins, which occur as a pentamer. Together with the fiber protein, it is responsible for the attachment of the virus to cell surfaces [9].

The capsid is stabilized by several minor scaffolding proteins: VI, VIII, IX, IIIa, and IVa2. Protein VI is located beneath the hexons in the viral capsid. When the virus enters the host cells, the lowered pH causes destabilization of the capsid, which liberates protein VI and promotes membrane disruption [10]. Proteins VIII and IX are thought to aid in stabilization and/or assembly of the virion. Protein IIIa is associated with the penton base and is critical to major late mRNA and protein expression [11]. Protein IVa2 plays a role in viral DNA packaging and is a transcriptional activator of the major late promoter. Research suggests that the IVa2 protein plays a role in viral DNA packaging and that a functional interaction between IVa2 and the rest of adenovirus packaging machinery is serotype specific [12].

Its genome carries five early genes: E1A, E1B, E2, E3, and E4 [14]. The E1A gene induces expression of other viral early genes. The E1B gene delays host cell lysis during viral replication and after replication is complete, it induces apoptosis and controls the export of viral transcripts. Deletion of E1 results in viruses that are severely impaired in their ability to replicate and allows for the insertion of approximately 5.1kb of new DNA [15].

Virus Replication

Adenoviruses replicate in the nucleus, and their replication is facilitated by extensive modulation of the host immune response. Viruses bind to host cell receptors via their Penton fiber knobs and subsequent internalization is mediated by the interaction between the Penton base and cellular integrins. The outer capsid is then removed and the core comprising the viral genome with its associated histones enters the nucleus where messenger RNA (mRNA) transcription, viral DNA replication, and assembly of virions occur. In the nucleus, the genome is transcribed by cellular RNA polymerase II according to a complex program involving both DNA strands [17].

Replication is divided into two phases: early and late. The late phase starts upon onset of DNA replication. Before and independently of genome replication, immediate early and early mRNAs are transcribed from the input DNA. Transcription of the Adenovirus genome is regulated by virus encoded transacting regulatory factors. Products of the immediate early genes regulate expression of the early genes. Early genes are encoded at various locations on both strands of the DNA [17].

There are five early (E) transcriptional units (E1A, E1B, E2, E3, and E4), two intermediate units (IX and IVa2), and one late (L) unit from which five families of late mRNAs (L1 to L5) are transcribed. Each early region is under the control of a separate promoter,

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whereas the late region uses a single promoter called the major late promoter. The E1A region of the viral genome encodes proteins that are essential for three main outcomes of early adenovirus transcription: Induction of cell cycle progression (DNA synthesis) to provide an optimal environment for virus replication; Protection of infected cells from host antiviral immune defenses, including cytokine induced apoptosis; Synthesis of viral proteins necessary for viral DNA replication [18].

Figure 1: Adeno virus structure (Russell, 2001) [13].

Figure 2: Genome organization of adenoviruses [16].
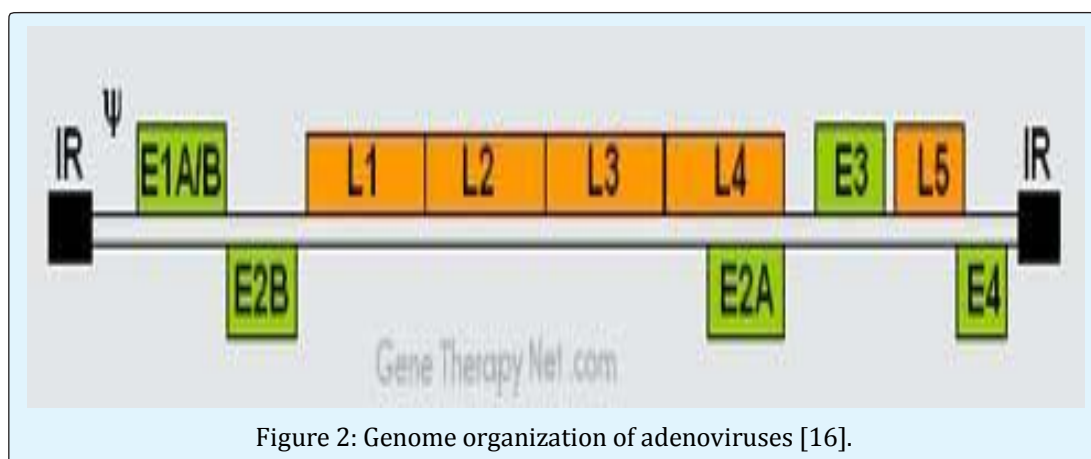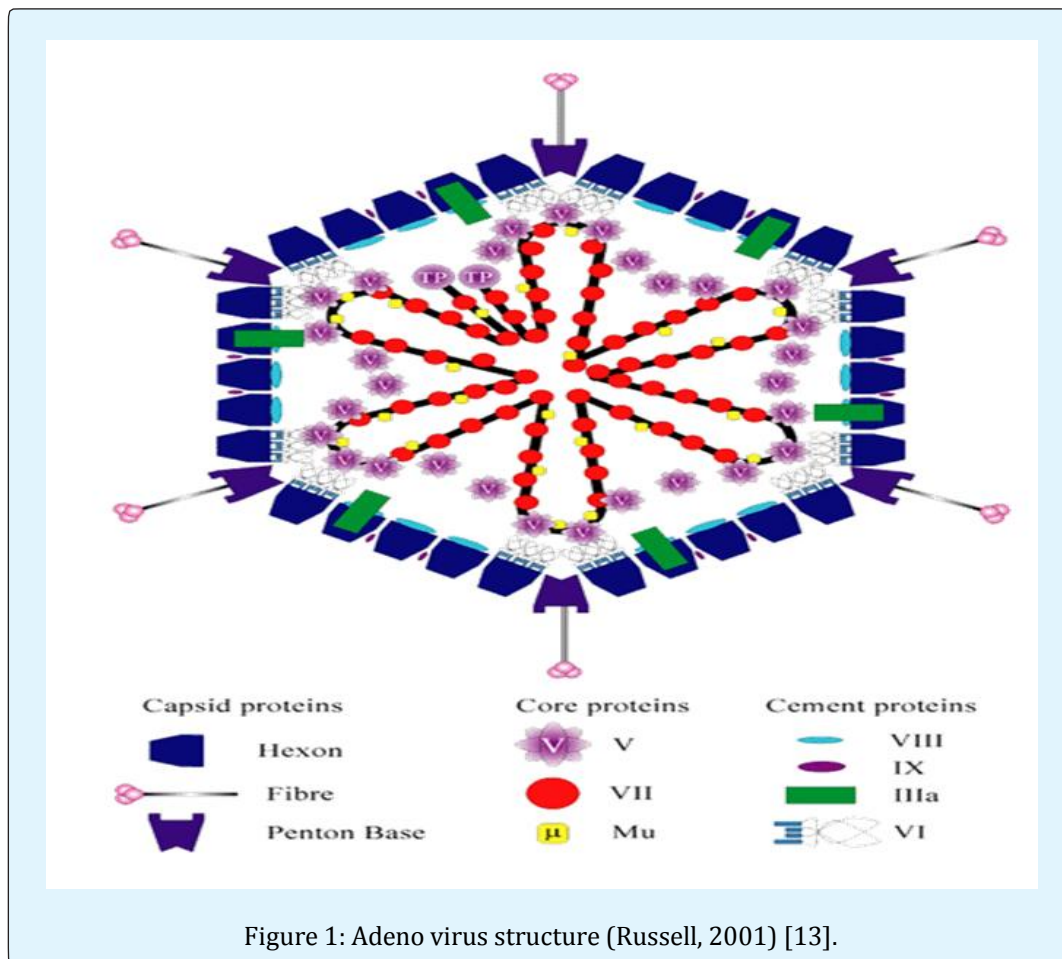
Adeno Virus as a Vaccine Vehicle

Construction of Adenoviral vectors

Numerous strategies have been developed to construct Adenovirus vectors carrying a foreign gene insert. Traditionally, Adenovirus vectors were constructed using two standard methods. The first method is the *in vitro* ligation method involving the ligation of a DNA fragment obtained by restriction digesting of a plasmid carrying the foreign gene insert flanked by Adenovirus sequences with a DNA fragment representing the rest of Adenovirus genome [19]. The second method consisted of homologous recombination in permissive cell lines between two plasmids. The shuttle plasmid which is carrying the foreign gene insert flanked by Adenovirus sequences for site specific insertion and the genomic plasmid carrying almost the entire Adenovirus genome [20].

Alternate approaches have been developed to avoid the limitations of traditional methods. One such strategy is based on the highly efficient homologous recombination machinery of *E. coli* (BJ5183). Adenovirus vectors homologous recombination between a linearized or intact plasmid containing almost an entire Adenovirus genome and a shuttle plasmid containing an exogenous expression cassette flanked by homologous sequences from the site of insertion in the Adenovirus genome generates an infectious clone with a modification and/or insertion in the desired region [21].

A similar strategy employing homologous recombination in yeasts has been reported. This strategy involves homologous recombination between Adenovirus DNA and the yeast artificial chromosome vector containing sequences from the left and the right termini of Adenovirus genome, resulting in the generation of a yeast artificial chromosome containing an infectious copy of Adenovirus genome. Transfection of the excised Adenovirus genome into appropriate cells results in generation of infectious virions [22].

Deletion: The adenoviral capsid allows some latitude regarding the length of genome that can be packaged efficiently. Foreign sequences of up to 1.8 kb can be inserted into the adenoviral genome without necessitating deletions. Nevertheless, to increase the permitted size of the insert and to modify the biology of adenoviral vectors, vaccine constructs are generally based on Adenoviral vectors with deletions of transcription units. The initial adenoviral vaccine vectors were deleted of E3, which encodes gene products that are nonessential for virus replication [23].

Most vectors currently used as vaccine carriers are deleted in E1 gene. This not only renders the vectors replication defective but also allows for sustained antigen presentation by reducing the vectors' ability to induce death of the infected cells. E1 is essential for viral replication and therefore has to be provided in Trans during production of the vectors. To incorporate larger foreign sequences, vectors deleted of E1 and E3 have been develop and they accommodate up to 7.5 kb of foreign DNA [24].

The original E1 deleted Adenoviral vectors were constructed by homologous recombination. Cells were co-transfected with purified Adenoviral genome deleted of E1 by restriction enzyme digest and a shuttle vector that contained the left handed ITR, the E1a enhancer, the encapsidation signal, the cytomegalovirus (CMV) IE promoter (or another suitable promoter), a multi cloning site for insertion of the gene of interest, and the SV40 poly (A) signal followed by sequences from the Adenoviral genome located 3' of the E1 domain. Now most E1-deleted Adenoviral vectors are generated from so-called molecular clones in which the entire Adenoviral genome including the ITRs is carried in a bacterial plasmid, allowing for its propagation in *Escherichia coli* [25] E3 deleted Adenoviral vectors are replication competent and thus less predictable than replication defective vectors. This raises concern about potential toxicity especially if they are used in immune compromised patients. It has been argued that replication competent denoviruses achieve higher antigenic loads compared to replication defective vectors and thus result in higher and more sustained transgene product-specific immune responses [26].

**Replication competent adenoviral vectors:** Replication competent adenoviral vectors encode all of the remaining adenoviral antigens in addition to the transgene product, i.e., The vaccine antigen. The adaptive immune response will thus be directed toward a multitude of different antigens, which can negatively affect the desired immune response to the vaccine antigen through competition for MHC binding sites or cytokines needed for activation, differentiation and proliferation of T and B cells. Furthermore, adenoviruses cause in most cell types lytic infections and, once the viral progeny has been assembled, result in apoptotic death of the infected cells. This shortens the duration of antigen presentation by individual infected cells, which in turn could have a negative effect on induction of CD8+ T cells, which are stimulated primarily by peptides derived from *denovo* synthesized antigens, while potentially favoring activation of CD4+T cells, which are induced by peptides derived from lysosomal cleavage of proteins taken up by phagocytosis or pinocytosis [24].

Replication incompetent adenoviral vectors: Replication incompetent vectors contain an antigen

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expression cassette substituted for the deleted E1A–E1B region. These replication incompitant adenoviruses can not replicate because of the deletion of the essential viral E1 gene region containing two genes, E1a and E1b. The removal of the E1 gene region creates room for the vaccine expression cassette and prohibits transactivation of viral genes required for viral replication. In addition to the E1 deletion, the viral E3 gene is dispensable for production of recombinant virus as well as the E4 gene, which reduces proinflammatory responses in vivo. Typically, the E3 region is also deleted to accommodate larger insertions. Regions of E2 and/or E4 can be deleted to diminish expression of late viral genes. Helper dependant vectors are deleted of all viral genetic information except the termini and the packaging sequence, which are required for vector propagation by helper systems. The antigen expression cassette is inserted into the deleted region together with a 'filler' sequence, so that the overall vector length is approximately the 36 kbp size similar to native Ad, to achieve efficient propagation [27]. Replication selective adenoviruses contain deletions in E1A or E1B, which render their propagation selective in cancer cells deficient in p53 or pRB function. Alternatively, tissue specific promoters can be substituted for the E1A promoter, so that productive virus infection occurs selectively in tumors having appropriate factors for activity of these promoters [28].

Figure: Adeno virus based vaccines [17].
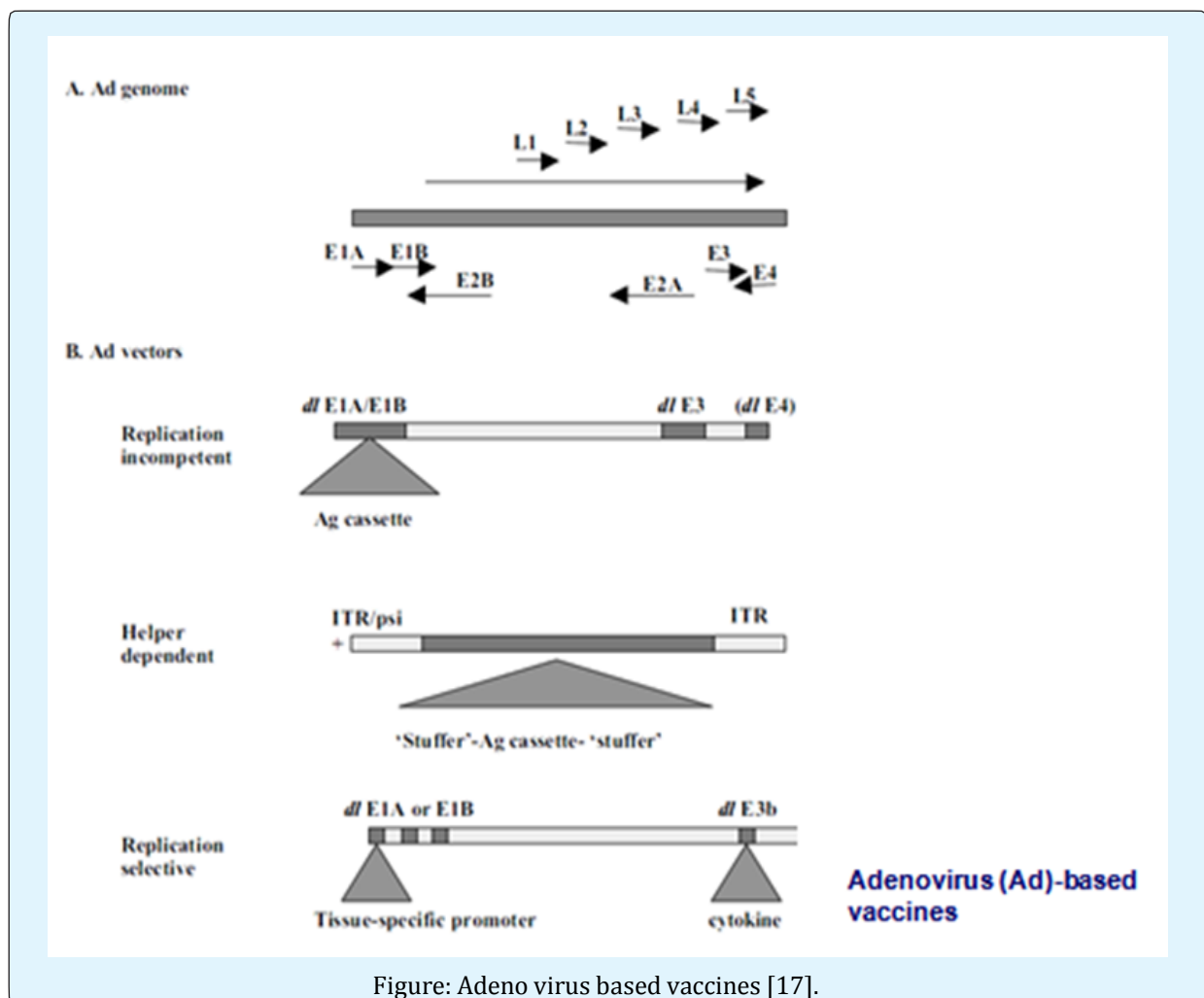
Adeno associated viral vectors: Adeno Associated Viruses are non enveloped, single stranded DNA viruses with a diameter of 18-25 nm. The virus particle is composed of an icosahedric capsid and one single molecule of the viral genome of either positive or negative sense. They belong to the Parvoviridae family and are classified in the Dependo virus genus. Adeno Associated Viruses are replication defective and require co infection with a helper virus, such as adenovirus to undergo a productive infection in the cultured cells. Adeno Associated Viruses are very resistant to extreme conditions of pH, detergent and temperature, making them easy to manipulate. The characteristic feature of Adeno Associated Virus is its deficiency in replication

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and thus the inability to multiply in unaffected cells. The first factor ascribed as providing successful generation of new Adeno Associated Virus particles, was the adenovirus, from which the name AAV originated. It has been shown that Adeno associated virus replication can be facilitated by selected proteins from the adenovirus genome [29].

The recombinant Adeno associated virus has attracted tremendous interest as a promising vector for gene delivery. These vectors are simple, versatile and safe and successfully used for the long term expression of therapeutic genes in animal models and patients. Furthermore, studies have demonstrated that recombinant Adeno associated vectors can evade the immune response and mediate a durable expression of transgene *in vivo* [30]. However, evidence has been gathering that in some circumstances, the Adeno associated virus vector may initiate adaptive immune responses to the transgene product [31].

Altered tropism: The tropism of Adenoviral vectors can be altered by genetic engineering of the fiber knob. Replacing a large portion of the C-terminus of HAd5 fiber with the σ1 protein of Reovirus type 3, both of which share the structural feature of forming trimers through triple β spiral motifs and a head and tail morphology results in stable vectors that use the Reovirus receptor junctional adhesion molecule 1 for viral entry and thus show improved transduction of certain cell types, including dendritic cells, expressing this receptor [32]. AdHu5 vectors pseudo typed with fibers from Adenoviruses of subgroups B, C, D, and F result in vectors with reduced tropism for myoblasts and endothelial cells (fibers from subgroups B and D) and increased tropism for dendritic cells (fibers from subgroup B, i.e., AdHu30 and AdHu35) *in vitro* and *in vivo* without affecting the resulting transgene product specific B or T cell responses [33].

In another report, the fiber of the subtype C AdHu5 virus was replaced with that of a subtype B fiber, i.e., the one derived from AdHu35 virus. Again, the pseudo typed vector has an increased ability to transduce dendritic cells and results in improved induction of CD8^{+} T cells *in vitro* or *in vivo* upon transfer of transduced dendritic cells [34].

Immune Responses Generated by Adenoviral Vectors

Both innate and adaptive immune responses are induced by adenoviral vectors and responses are generated against the transgene but also the vector itself. Following systemic administration, adenovira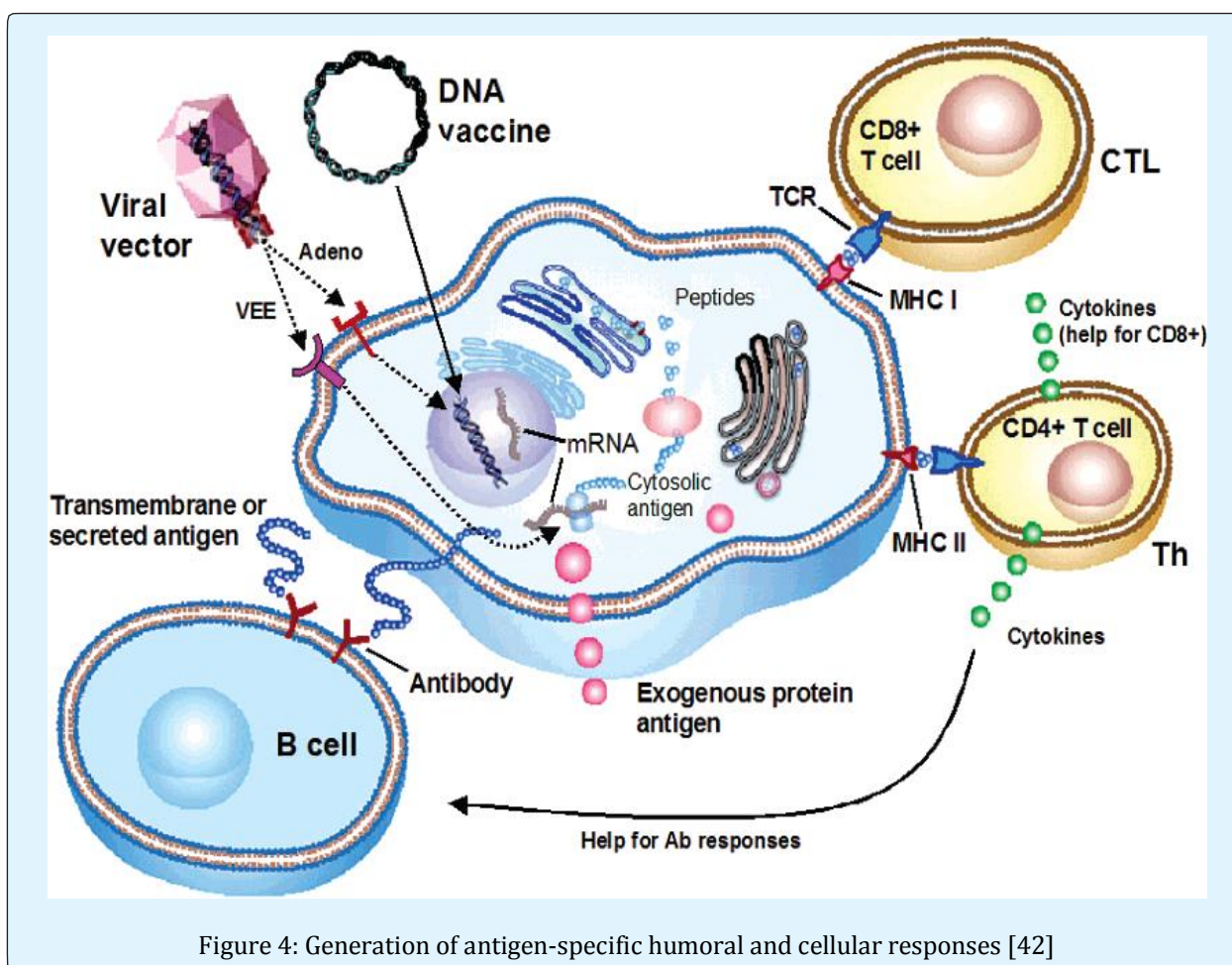l vectors will attach to the Coxsackie Adenovirus Receptor (CAR) on host cells and are internalized. Initially entering the cell within an endosome the virus subsequently escapes from the endosome and from within the cytoplasm the viral DNA will enter the nucleus of the cell and is eventually transcribed [35]. Immune responses can be triggered by internalized viruses via various Toll-like receptors [36].

Adenoviruses are highly immunogenic. They activate the innate immune system presumably by expressing so called pathogen associated molecular patterns (PAMPs). PAMPs bind to pathogen recognition receptors on host cells, including those of the innate immune system, thus initiating production of pro inflammatory cytokines and differentiation of immature dendritic cells into professional antigen-presenting cells. Systemic administration of high doses of AdHu5 vectors into mice or monkeys was shown to trigger rapid release of IL-6, IL-12, and TNF-ɑ and accumulation of transduced macrophages and dendritic cells in lymphatic tissues [37].

Adaptive immune responses are directed to both early and late antigens of Adenovirus. Virus neutralizing antibodies induced by Adenoviral infections or upon Adenoviral vector delivery are primarily directed against the surface loops of the viral hexon although antibodies to the penton base or the fiber can also neutralize Adenovirus. CD4^{+}T cells, which are mainly of the Th1 type, cross-react between different Adenoviral serotypes. In experimental animals' Adenoviral vectors induce CD8^{+} T cell responses to different structural proteins [38].

The potential for Adenoviruses to elicit powerful B cell and T cell responses in the mammalian host are the main reason for the use of these vectors in vaccine development. The strong immune responses elicited by these vectors have been linked to their ability to infect immature dendritic cells thereby activating them to become mature antigen presenting dendritic cells thus promoting T cells responses [39].

The binding of the Adenovirus fiber to the CAR receptor leads to the production of proteins such as P13K kinase and junctional adhesion molecule like protein causing the production of various chemokines [40]. Significant levels of cytokines including IL-6 and TNFα are usually detected within 24 hours following Adenoviral vector administration. Importantly even when the vectors have few or no Adenoviral genes they still elicit significant innate responses characterized by up regulated neutrophils and macrophage activity at the site of vector administration which are induced by the viral capsid [41].

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Figure 4: Generation of antigen-specific humoral and cellular responses [42]

Adenoviral Vectors Currently Used in Veterinary Vaccines

The appeal of adenovirus based vaccine vectors stems from their use as efficacious vaccines. Most studies to date have focused on human serotype adenoviruses, particularly human adenovirus type 5. Human serotype adenovirus vaccine vectors are particularly useful for development of veterinary vaccines as neutralizing antibodies to the vector will not usually be present in the vaccinates and to date a range of veterinary vaccine candidates have been developed using this platform [43]. Adeno viruses from various species have been developed as vectors and some examples are listed in Table 1.

Vector	Animal Model	Route of delivery	Results of animal studies	Reference
Canine adenovirus serotype 2/ rabies virus glycoprotein	Dog	Subcutaneous	100% protection following challenge with rabies virus	Hu et al., 2006 [44]
Human adenovirus serotype 5/avian influenza virus haemagglutinin (HA)	Chicken	Subcutaneous	High levels of HA specific antibodies; High levels of IFN-gamma	Ramos et al. 2010 [45]
Replication defective Had 5 containing capsid polypeptide from FMD V	Pig	Intramuscular	Sufficient protection	Mayr et al., 1999 [46]
Fowl adenovirus serotype 1/ VP2 protein of infectious bursal disease virus	Chicken	Oro nasal	Complete protection	Francois et al., 2004 [47]

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Replication defective HAd 5 expressing HA from swine influenza virus	Pig	Subcutaneous	Induce both cellular and humoral response	Wesley et al., 2004 [48]

Table 1: Examples of adenoviruses that have been developed as veterinary vaccine vectors

The Potential for Commercial Adenoviral Vector Based Veterinary Vaccines

Conventional vaccines have played an important role in the control and possible eradication of certain diseases e.g. rinderpest. However, new approaches to vaccine development are urgently required for those diseases where efficacious vaccines do not exist [49]. Despite years of research into the technologically advanced DNA and viral/bacterial vector technologies, conventional vaccines based upon either killed or attenuated pathogens or recombinant antigens still form a major part of the veterinary vaccine market today. One of the main reasons for this is the relatively long process leading to commercialization of a new vaccine which involves establishing vaccine efficacy and safety prior to obtaining registration, which may be complicated if the vaccines were to be commercialized globally [50].

Currently however, veterinary vaccines comprise a fraction of the market size of human vaccines; consequently there are much lower levels of investment into veterinary vaccine research and development. These issues can have a negative impact on the utilization of new technologies in veterinary vaccine development. The commercialization of vaccines whether for human or veterinary use usually begins following extensive testing of the vaccine candidate in animal models and field trials? The final stage of this arduous process would be market authorization which for a veterinary vaccine would involve obtaining permission from veterinary authorities to allow the vaccine to be made available to the relevant consumers [51].

Over the years a number of adenoviral vector based veterinary vaccines have been developed but none are currently licensed. One such Adenoviral vector based veterinary vaccine is currently under consideration for commercialization. This is a replication defective human adenovirus serotype 5 containing the capsid and 3C protease coding regions of foot and mouth disease virus as a vaccine candidate has been developed and gone through field trials and other testing in preparation for commercial production [52].

Challenges in Application of Adenoviral Vaccine Vector

Despite the many advantages of this technology there are some potential drawbacks to consider, such as the potential toxicity of adenoviral vaccine vectors. For example, viral based vectors elicit an inflammatory cytokine response (by stimulating both innate and adaptive immune responses), thus promoting harmful side effects in the host. In light of this, these viral vaccine vectors must be thoroughly evaluated for potentially harmful immunological reactions on the host immune system, especially in the context of pre-existing immunity to the vaccine vector prior to conducting field trials [53].

Adenoviral vector based systems are clearly underutilized in veterinary vaccine development despite being well characterized and shown to be potent inducers of protective immune responses. If these vectors are to be used widely to construct veterinary vaccines that can be commercialized, more research needs to be undertaken on this platform, particularly studies focusing on the comparison of this platform with other viral vector platforms and the use of adenoviral vaccines in prime boost immunization protocols with non Adenoviral vector based vaccines [54].

Other important issues to be considered when developing Adenoviral vector based on vaccines are the choice of transgene and the stability of the vector genome. Ideally extensive research to identify the most immunogenic antigens of a pathogen should therefore be undertaken before vaccine development begins. In order for a vaccine to be protective, the pathogen specific transgene used in the construction of the vaccine should contain highly immunogenic epitopes otherwise the immune responses generated by the vaccine will be suboptimal. Studies conducted to ensure the stability of the viral vector genome must also be conducted soon after the vaccine is constructed and well before large scale production of the vaccine as it has been reported that mutants which do not express the vaccine transgene can randomly arise when producing large quantities of viral vector based vaccines [55].

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Due to the ubiquitous nature of Adenovirus, a large percentage of animal population has variable levels of neutralizing antibodies know as 'preexisting vector immunity' or 'vector immunity' against more than one Adenovirus serotypes. These neutralizing antibodies are directed against the viral capsid components and adversely affect the uptake of Adenoviral vectors by target cells. Adenovirus neutralizing antibodies persist for years posing a challenge when repeated administrations are necessary [56].

Preexisting immunity due to natural infections results in sustained virus neutralizing antibody titers, a major handicap for the successful use of common serotypes of Adenovirus, as carriers for gene replacement therapy or vaccine carriers. Neutralizing antibodies even if present at moderate titers reduce uptake of the Adenoviral vectors by cells, including antigen presenting cells [57].

Conclusion and Recommendations

Advances in molecular virology in concert with viral immunology now allow for the genetic engineering of vectors expressing solely those viral antigens that induce immune correlates of protection. Adenoviruses have moved to the forefront of vaccinology and are showing substantial promise as vehicles for antigen delivery for a number of vaccines currently being developed. Due to the versatility and variety of Adenovirus serotypes, they will be valuable tools for developing vaccines against new pathogens and against those to which vaccines have yet to be discovered. Most vectors currently used as vaccine carriers are deleted in E1. This renders the vectors replication defective, to increase the permitted size of the insert and also allows for sustained antigen presentation by reducing the vectors' ability to induce death of the infected cells. The potential for Adenoviruses to elicit powerful B cell and T cell responses in the mammalian host are the main reason for the use of these vectors in vaccine development. Preexisting Adenoviral vector immunity has been shown to decrease the immunogenicity of Adenoviral based vaccines in animal models. Several strategies to overcome this limitation are being explored and have shown promise in pre-clinical studies. One such strategy involves the use of vectors derived from human Adenoviruses specially HAd 5. Based on the above conclusion the following recommendations are forwarded:

- Adenoviral vaccine vectors must be thoroughly evaluated for potentially harmful immunological reactions on the host immune system.
- Extensive research on Adeno viral vaccine vectors should be undertaken for effective utilization and commercialization of veterinary use.

- The time taken for licensing and commercialization process of vaccine should be minimized.
- Application of such new vector based vaccine technology should be promoted in our country.

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