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Review

Bovine adenovirus-3 as a vaccine delivery vehicle

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ABSTRACT

The use of vaccines is an effective and relatively inexpensive means of controlling infectious diseases, which cause heavy economic losses to the livestock industry through animal loss, decreased productivity, treatment expenses and decreased carcass quality. However, some vaccines produced by conventional means are imperfect in many respects including virulence, safety and efficacy. Moreover, there are no vaccines for some animal diseases. Although genetic engineering has provided new ways of producing effective vaccines, the cost of production for veterinary use is a critical criterion for selecting the method of production and delivery of vaccines. The cost effective production and intrinsic ability to enter cells has made adenovirus vectors a highly efficient tool for delivery of vaccine antigens. Moreover, adenoviruses induce both humoral and cellular immune responses to expressed vaccine antigens. Since nonhuman adenoviruses are species specific, the development of animal specific adenoviruses as vaccine delivery vectors is being evaluated. This review summarizes the work related to the development of bovine adenovirus-3 as a vaccine delivery vehicle in animals, particularly cattle.

1. Introduction

Adenoviruses (AdVs) are non-enveloped double stranded DNA viruses with icosahedral capsid symmetry composed of 252 capsomers. The genome size ranges between 24 and 45 kb [1,2]. Although adenoviruses were first identified in the early 1950s from the adenoids of humans with acute respiratory infection [3,4], today, there are over 120 serotypes, isolated from different species, including, mammals, birds, reptiles and fish. While adenoviruses can infect a wide variety of animals, including humans, birds and livestock, they are usually species-specific [2,5,6]. AdVs have been proposed to be clustered in five phylogenitically distinct groups namely; *Mastadenovirus*, *Aviadenovirus*, *Atadenovirus*, *Siadenovirus* and *Ichtadenovirus*, with each virus group having a distinct and characteristic genome structure [7,8]. The overall genetic organization of members of the *Mastadenovirus* including human adenovirus (HAdV)-5 [9], canine adenovirus (CAdV)-2 [10], Simian adenovirus (SAdV) [11], bovine adenovirus (BAdV)-3 [12], porcine adenovirus (PAdV)-5 [13] and PAdV-3 [14] appears to be conserved. The *Mastadenovirus* genome is divided into five discrete transcriptional units. The early regions E1 and E4 are located at either end of the genome, while E2 and E3 are separate and internal. The central core region constitutes the late region. In addition, there are two delayed early transcriptional units named IVa2 and pIX. Despite this genetic conservation, some regions are highly divergent (based on structure of transcripts and proteins) among different members of *Mastadenoviruses*. In particular, the deduced amino acid sequence of ORFs in the E1, E3 and E4 regions of BAdV-3 show little or no homology to the corresponding proteins encoded by genomes of other members of *Mastadenoviruses* [12]. Since these proteins are involved in virus cell interaction and viral gene expression, variations in these proteins may reflect the diverse host range and pathogenic potential of different members of *Mastadenoviruses.*

In recent years, much attention has been focused on evaluating adenoviruses as viral vectors due to their ability to infect both dividing and non-dividing cells, capacity to package large foreign genes, relative ease to produce high titer recombinants in cell culture [15], elicit strong antigen specific T cell responses and lack of virulence [16,17]. Although recombinant HAdVs have been proven to deliver vaccine antigens to domestic animals [18–21] and birds [22,23], regulatory concerns regarding safety has limited their use in domestic animals. Moreover, species specificity limiting host range, restricted replication in non host species and

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stability of nonhuman adenoviruses has led to the evaluation of animal [CAdV-2 [24], PAdV-3 [25], PAdV-5 [26], BAdV-3 [27,28] and poultry (Fowl adenovirus (FAdV) [29] specific adenoviruses as vaccine delivery vectors. Since BAdV-3 is a natural nonpathogenic virus with restricted host-range, grows to high titers and can be delivered intranasally without affecting the meat quality of food producing animals, BAdV-3 is being evaluated as vaccine delivery vector in animals including cattle.

2. Molecular biology of bovine adenovirus-3

BAdV-3 is 75 nm in diameter, non enveloped icosahedral particle containing a double stranded DNA genome. The genome of BAdV-3 is 34, 446 base pairs [12] flanked on either end by 195 bp inverted terminal repeats (ITRs) [30], which play a key role in DNA replication. Unlike other adenoviruses, the ITRs of BAdV-3 are longer and contain a high GC content [12]. Like other adenoviruses, the packaging domain is located in the left end of the viral genome overlapping the transcriptional control region of E1A, but E1A region expression distinctively appears to be essential for packaging in BAdV-3 [31,32]. Though the TATA or CAAT boxes are absent in the regions between the left ITR and upstream of the E1A start codon, the expression of the E1A open reading frame is driven by a promoter located within the ITR [33].

Based on the transcriptional analysis [12,34–37], BAdV-3 genome appears to be organized (Fig. 1) into early, intermediate and late regions [12]. Although genome organization appears similar to other members of *Mastadenoviruses*, the structure and function of some proteins encoded by different regions of BAdV-3 appears to be different [38–44].

3. Development of BAdV-3 as a vector for vaccination

3.1. Method for isolation of recombinant BAdV-3

Initial attempts to generate recombinant BAdV-3 using homologous recombination in Madin Darby bovine kidney (MDBK) cells co-transfected with purified BAdV-3 genome and a transfer vector containing a deletion in E3 region were inefficient and mostly unsuccessful [45–47] due to low transfection efficiency and inefficient recombination in MDBK cells. However, two improvements namely availability of homologous recombination machinery of *Escherichia coli* [48] and use of bovine retina cells for transfection of restriction enzyme excised modified BAdV-3 genome from plasmids has facilitated the efficient generation of recombinant BAdV-3 [47,49]. Recently, a more efficient and time saving method of generating recombinant BAdV-3 has been reported by transfection of endonuclease *I-SceI* expressing non bovine (cotton rat lung) cells with circular BAdV-3 genomic DNA flanked by *I-SceI* recognition site [50].

3.2. Sites for insertion of foreign genes

3.2.1. E1 region

Isolation of replication competent BAdV-3 containing partial deletion of $E1B_{small}$ suggested that this region is not essential for replication of BAdV-3 [51] in fetal bovine retina cells and may be used for foreign gene insertion. However, viable recombinant BAdV-3 with E1A [52–54] or $E1B_{large}$ deletion [33,52] could not be rescued suggesting that these regions are essential for replication of BAdV-3. However, packaging cell lines expressing E1 region of BAdV-3 (FBK-34; MDBK-221; [53]) or HAdV-5 (VIDO R2, FBRT-HE1; BHH3 and BHH8 [53,54] supported the replication of only E1A (541 bp) deleted BAdV-3 (Fig. 2) and not complete E1 region deleted BAdV-3 [52]. Replication-defective BAdV-3s containing insertion of 2.3 kb of foreign DNA in E1A region of E1-E3 deleted BAdV-3 could be isolated only in E1A complementing cell lines [52].

Since replication-defective BAdV-3 vector cannot undergo multiple replication cycle and therefore are less likely to spread to the environment, they are considered safer. One of the major concerns with regard to the use of live viral vectors for vaccination in animals is the release of potential recombinants into the environment. However, since the replication-incompetent recombinants undergo an abortive infection, the level of foreign gene expression is lower than that obtained using replication-competent BAdV-3.

3.2.2. E3 region

The E3 region of BAdV-3 is 1.591 kb. Since E3 region is non essential for replication of adenoviruses [46,55], initial attempts resulted in isolating a viable recombinant BAdV-3 containing deletion of 1.245 kb of E3 region [46]. Although potential insertion capacity of E3 deleted vector is 3 kb [46], the insertion of 2.8 kb foreign DNA [56] has been successful in generating viable replication-competent recombinant BAdV-3. The foreign open reading frames (ORFs) inserted in E3 are efficiently expressed using upstream endogenous (E3/MLP) promoters [28,46,56,57] or exogenous promoters [56,57]. However, insertion of foreign ORFs antiparallel to E3 transcription does not lead to the generation of viable recombinant BAdV-3. Moreover, addition of exogenous consensus sequence for polyadenylation of foreign ORF affects the replication efficiency of recombinant BAdV-3 (Lobanov and Tikoo, unpublished data). Interestingly, efficient expression of a RNA virus required addition of an intron and consensus sequence for polyadenylation upstream and downstream, respectively, of the gene [55]. Since E3 deleted recombinant BAdV-3 are replication competent (Fig. 2), less amount of virus may be required to induce a protective immune response.

3.2.3. E4 region

The first site for insertion of foreign ORF has been identified in a transcriptionally inactive region between start of E4 and right ITR of BAdV-3 [12,34]. Isolation of replication competent BAdV-3 containing insertion of 1.9 kb foreign DNA at nucleotide (34059) suggested that this region is non-essential for BAdV-3 replication (Fig. 2). However, exogenous promoter and consensus sequence for polyadenylation are required for expression of the foreign ORF.

The other sites for potential insertion of foreign ORF have been identified in transcriptionally active E4 region of BAdV-3 [58]. Isolation of replication competent BAdV-3 containing deletion of N-terminus 1.501 kb or C-terminus 1.342 kb (Fig. 2) in E4 region suggested that these regions are not essential for replication of BAdV-3 [58], thus increasing the potential insertion capacity of E3–E4 deleted BAdV-3 to 4.5 kb [58].

4. Evaluation of recombinant BAdV-3 as vaccine delivery vector

4.1. Recombinant BAdV-3 expressing single antigen

Much of the initial efforts of developing replication-competent BAdV-3 based vectors have focused on non-essential E3 region. Earlier, successful expression of a reporter ORF inserted in partially deleted E3 region of BAdV-3 [45] suggested the feasibility of utilizing E3 region for expression of foreign genes. Thus, subsequent expression of different forms of (BHV)-1 vaccine antigen gD ORF [46], (BVDV) E2 ORF [57] or (BCV) virus HE ORF [55] inserted individually in fully deleted E3 region of BAdV-3 demonstrated the potential of developing BAdV-3 based recombinant vaccines for cattle. These ORFs were selected as earlier reports have suggested the potential of bovine herpesvirus (BHV)-1 glycoprotein gD [59], bovine viral diarrhea virus (BVDV) E2 glycoprotein [60] or bovine