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Immune responses to recombinants of the South African vaccine strain of lumpy skin disease virus generated by using thymidine kinase gene insertion

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Abstract

The South African vaccine strain of lumpy skin disease virus (type SA-Neethling) is currently being developed as a vector for recombinant vaccines of economically important livestock diseases throughout Africa. In this study, the feasibility of using the viral thymidine kinase gene as the site of insertion was investigated and recombinant viruses were evaluated in animal trials. Two separate recombinants were generated and selected for homogeneity expressing either the structural glycoprotein gene of bovine ephemeral fever virus (BEFV) or the two structural glycoprotein genes of Rift Valley fever virus (RVFV). Both recombinants incorporate the enhanced green fluorescent protein (EGFP) as a visual marker and the *Escherichia coli* guanine phosphoribosyl transferase (gpt) gene for dominant positive selection. The LSDV-RVFV recombinant construct (rLSDV-RVFV) protected mice against virulent RVFV challenge. In a small-scale BEFV-challenge cattle trial the rLSDV-BEFV construct failed to fully protect the cattle against virulent challenge, although both a humoral and cellular BEFV-specific immune response was elicited.

Keywords: Capripoxvirus; Recombinant vaccine; Lumpy skin disease virus; Rift Valley fever; Bovine ephemeral fever

1. Introduction

The development of poxviruses as vectors for producing recombinant vaccines is well documented [1–3]. Although vaccinia virus (VV) was the first and most extensively developed poxvirus vector, concerns over its use in immunocompromised persons has led to the search for other poxviruses which might prove more suitable as vectors. Avipoxviruses undergo abortive replication in non-avian cells, yet can achieve expression of extrinsic gene products [4]. A canarypox-rabies glycoprotein recombinant vaccine was shown to induce both a humoral and cell-mediated immune response in human volunteers without adverse side-effects [2].

Capripoxviruses (type member sheeppox virus) are also being targeted as they are highly host-range restricted and a number of attenuated strains have been used effectively as vaccines for many years [5,6]. A number of recombinants using the northern African KS-1 strain of capripoxvirus as vector have already shown tremendous potential [7,8]. In southern Africa only one member of the *Capripoxvirus* genus is represented, namely lumpy skin disease virus (LSDV) of cattle. Recent sequence data have revealed that although northern and southern African field strains of LSDV are very similar, there are vast genomic differences between these and the highly cell-passaged and attenuated South African vaccine strain [9,10]. Due to its high level of host-range restriction and history of safe use in the field, as well as its ability to provide long-term immunity, this South African vaccine strain of LSDV is also being targeted for development as a vector for recombinant vaccines for use in

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the veterinary field. A number of potential insertion sites have been identified with some of these showing good results [11].

Our laboratory chose the viral thymidine kinase (TK) gene as the site for insertion of foreign genes as this gene has proven non-essential for other poxviruses such as VV [12]. An added advantage in targeting this site is the possibility of using the resulting TK-negative phenotype as a means to select for recombinants without the need for additional selection markers [1]. However, we have previously shown that although the TK gene appears suitable as a site for the insertion of foreign genes, the virus is still dependent on some TK activity for normal growth [13]. For the successful generation and selection of recombinants, we thus make use of the viral TK gene as the insertion site and on cellular TK activity from primary foetal bovine testes cells to help sustain viral replication. Selection of recombinant over wild-type (wt) virus is then achieved through use of the *Escherichia coli* gpt dominant selectable marker gene [14]. In addition, we have included the enhanced version of the green fluorescent protein (EGFP) gene from the jellyfish *Aequorea victoria* as a visual marker to assist with the selection of homogeneous recombinants [15].

Bovine ephemeral fever virus (BEFV) is an arthropod-borne single-stranded negative sense RNA virus belonging to the family *Rhabdoviridae* (genus *Ephemerovirus*) [16]. It causes an acute febrile disease in cattle and water buffalo. Neutralising antibodies have been found in other species of African wildlife [17]. Bovine ephemeral fever (BEF) is widespread throughout Africa, Australia, and parts of the Far East and is commonly known as “ephemeral fever” or “three-day stiffness sickness” due to the immobilisation of infected animals for 3–5 days following the height of viraemia and fever [18,19]. Although recovery may be complete, mortality occurs in 2–3% of cases and a permanent drop in milk production in cows and reduced fertility in bulls often occurs resulting in heavy economic losses [19]. There is a need to replace current live-attenuated virus vaccines due to heat sensitivity, especially in Africa where maintenance of a continuous cold-chain is extremely difficult.

Rift Valley fever (RVF) is a zoonosis with symptoms in humans ranging from mild influenza-like illness to severe complications such as ocular sequelae, encephalitis or haemorrhagic disease, sometimes resulting in death [20,21]. In ruminants the disease is usually mild but may cause severe disease in cattle, sheep and goats, especially in neonates. Generally considered a disease of sub-Saharan Africa, in more recent years it has spread throughout northern Africa to Saudi Arabia and Yemen [22,23]. Rift Valley fever virus (RVFV) belongs to the *Phlebovirus* genus of the family *Bunyaviridae* and consists of a three-segmented, single-stranded negative-sense RNA genome [24]. A number of live-attenuated and formalin-inactivated vaccines against RVFV have been in use for many years, although in a proportion of pregnant sheep vaccinated with the live-attenuated Smithburn vaccine, the vaccine appears to be responsible for causing abortions or teratology of the foetus and *hydrops amnii* and prolonged gestation in the dam [25]. There is thus also a need for alternative RVFV vaccines.

The southern African vaccine stain of LSDV is an excellent candidate as a vector for use in developing recombinant vaccines against these viruses (BEFV and RVFV). It is highly host-range restricted [26], stable [13], and has the ability to provide dual protection when used in cattle (against LSDV) and possibly even against sheep pox and goat pox in sheep and goats due to the capripoxviruses sharing a major surface antigen [27,28].

Two recombinants, expressing the structural glycoprotein genes of BEFV and RVFV, respectively, were generated. Inoculation of both recombinants into rabbits elicited the production of neutralising antibodies, and, in this paper we report on the immune responses and protective abilities of these recombinants in cattle (BEFV) and mice (RVFV).

2. Materials and methods

2.1. Viruses and cells

The South African vaccine strain of LSDV (type SA-Neethling) was used in the generation of the recombinants and as control virus. The vaccine was developed from a virulent field isolate over 40 years ago by 61 serial passages in lamb kidney cell monolayers followed by 20 passages in the chorioallantoic membranes of embryonated chicken eggs, and another three passages in lamb kidney cells [29]. The vaccine stock used for generating the recombinants was passaged a further 10 times in Madine-Darby bovine kidney cells and five times in foetal bovine testes (FBT) cells.

FBT cells were used for generation and selection of the recombinants and were grown in a 1:1 combination of Dulbecco’s modified Eagle’s medium (DMEM) and Ham’s F12 medium containing 8% foetal calf serum (FCS) and antibiotics (100 μg/ml penicillin, 100 μg/ml streptomycin and 250 μg/ml amphotericin) (all cell culture media reagents and supplements from Highveld Biological Products, SA).

The BEFV challenge material was virus-infected blood passaged once in a cow from a field isolate collected near Skeerpoort (border of Gauteng and North-West Province of South Africa) (isolate 1741), and the Rift Valley fever virus challenge strain was a mosquito isolate (no. AR 20368) passaged eight times in Madin–Darby bovine kidney cells and twice in hamsters (intra-peritoneal route).

2.2. Generation of recombinant viruses

The recombinant viruses were generated as described using the MPA-selection approach [6] except that the transfections were performed using Effectene (Qiagen, Germany) in place of DOTAP according to the manufacturer’s instructions. The structural glycoprotein gene of BEFV [30] and