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DNA vaccination with the Aleutian mink disease virus NS1 gene confers partial protection against disease

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Abstract

Aleutian disease virus (ADV) causes severe losses in mink. This happens in nature as well as in farms. In spite of several attempts to provide an efficient protective protein based vaccine, experiments have failed so far. Only partial protection has been obtained. The aim of this work was to construct and test a protective DNA vaccine based on the gene encoding for the ADV non-structural protein 1 (NS1) and to test this construct as a potential vaccine candidate against ADV infection or disease. First, the vaccine construct was tested by in vitro transfection studies. NS1 protein expression was found by immunofluorescent studies and the expected size of translated protein confirmed by Western blot. Then, 18 female mink were divided into three groups: a control group, a DNA vaccinated group, and a group which received DNA vaccine plus a boost with recombinant NS1 protein in the last immunization. After virus challenge, the two DNA vaccinated groups induced higher antibody levels in the first 23 weeks of the 32 week observation period. One month after virus challenge, the most interesting finding was, that the "DNA + protein" group exhibited a significantly higher percentage of $CD8^+$ cells, when compared to the levels in the two other groups. This, we believe, indicate a memory CTL response created by the vaccination. Most $CD8^+$ cells were found to contain interferon gamma as measured by FACS intracellular staining. Severity of Aleutian disease was judged by quantification of plasma gammaglobulin levels and mink death statistics. The findings let us to conclude, that the two DNA vaccinated groups of mink did show milder disease characteristics, but that the vaccine effect also in this trial could only be characterized as partial.

Keywords: Aleutian disease virus; Mink; DNA vaccination; Gammaglobulinemia; CD8; Flow cytometry

1. Introduction

Aleutian disease virus (ADV) is an autonomous parvovirus [10] that causes persistent and progressive infection in the adult mink (for reviews see [1,8,35]). The resulting disease, Aleutian disease (AD) or mink plasmacytosis, is characterized by development of hypergammaglobulinemia [4,9,36], increased number of $CD8^+$ lymphocytes [2,3,13] and organ infiltrations of mononuclear leucocytes [18,31]. The infection normally leads to development of immune complex diseases such as arteritis and glomerulonephritis [32,34] and may have a fatal outcome.

Infection with ADV elicits a strong humoral immune response resulting in high levels of antibodies directed against both capsid and non-structural viral proteins [3,4,9,11,36,37] as well as of autoantibodies [28]. However, none of these antibodies have the capacity to neutralize the virus [3,31]. On the contrary, an antibody-dependent enhancement of infection and disease has been observed [3,7,8,26,33].

Cell mediated immunity to ADV has only been demonstrated in few reports. The ADV specific lymphocyte transformation in peripheral blood lymphocytes was found to be very poor in persistently infected mink but present in non-persistently infected mink [6]. In another study using lymph

Abbreviations: ADV, Aleutian disease virus; AD, Aleutian disease; Ig, immunoglobulin; wpi, weeks post infection; PBLs, peripheral blood leucocytes; PBMCs, peripheral blood mononuclear cells; FACS, fluorescence activated cell sorter; BSA, bovine serum albumin

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node cells, Race et al. [38] found that ADV infected mink generated an antiviral lymphocyte response during the course of Aleutian disease. Only in the terminal stage of AD the cell mediated immunity was found depressed. In a recent study from our group [25] high levels of IFN-gamma and IL-4 producing $CD8^+$ cells during the course of infection has been demonstrated. These findings were taken as an indicator of active CD8 induced immunity in the AD affected mink, which however was not sufficient to prevent persistent infection.

No effective vaccine against ADV infection is currently available, although vaccination with recombinant NS1 protein has demonstrated a partial vaccine effect [3]. Genetic vaccines present numerous advantages over conventional vaccines due to their capacity to simulate a natural infection (presentation of peptides via both MHC I and II). Besides, they do not cause an infection risk. They facilitate the use of combination vaccines, they are stable at low and high temperatures and they normally provoke good memory responses [17,39,41,43]. DNA vaccination has been proven to be successful in the prevention of different infectious diseases in animals [16,21,22,29] including creation of strong cytotoxic immune responses [12,19,23,42]. Sometimes a "prime-boost" strategy has been necessary in order to obtain protection [27,40]. The aim of this work was to study the response to a newly constructed DNA vaccine based on the gene encoding the ADV non-structural protein NS1 and to test it against ADV infection of mink.

2. Materials and methods

2.1. Animals

Eighteen non-Aleutian genotype female mink (*Mustella vison*) were included in the study. They were 12 months old and had been vaccinated against canine distemper virus (CDV) 3 months before the beginning of the study. Mink were arranged randomly into three groups, housed in separate cages and fed a standard mink diet.

Blood was obtained by toenail cutting [24], collected in heparinized capillary tubes and processed immediately for further analyses. Plasma was collected and stored at −20 °C until used. Macroscopic pathological examinations were performed on all dead mink in order to assess any indication of severe Aleutian disease (enlarged mesenteric lymph node, splenomegaly, coloration and enlargement of the kidneys and hepatic leukocyte infiltration as indicated by white spots in the liver).

Experimental procedures were undertaken in accordance with the requirements of the Danish Animal Care and Ethics Committee.

2.2. DNA vaccine construction

Plasmid pNS1 was constructed by subcloning the NS1 coding sequence of ADV [14] into the *Xba*I site of pVR1012 vector. The pVR1012 plasmid and the NS1 containing plasmid were kindly donated by Vical Inc., San Diego, CA, USA and Dr. J. Christensen (Univ. of Copenhagen, Denmark), respectively. We used pVR1012 as a negative control vector. pNS1 and pVR1012 plasmids were subsequently transformed in *E. coli*, propagated and purified using EndoFree plasmid Giga kit (QIAGEN, Germany) following the instructions provided by the manufacturer. In order to obtain a vaccination grade plasmid DNA the following steps were added to the protocol: plasmids were resuspended O/N at 4 °C in endotoxin-free water, precipitated and washed in 70% ethanol and finally resuspended O/N at 4 °C in endotoxin-free PBS. Plasmid concentration was adjusted to 1 mg/mL using endotoxin-free PBS and kept at −20 °C until used.

2.3. Vaccination protocol

The eighteen mink were randomly divided into three groups: the "control" group ($n = 6$) which received pVR1012, the "DNA only" group ($n = 6$) which received pNS1 and the "DNA + protein" group ($n = 6$), which received pNS1 and a boost with recombinant NS1 protein [14] in the last immunization. Mink were injected three times (21-day intervals) with 800 μg of plasmid before virus challenge. The administration was partly intramuscularly (533 μg), given at 4–5 sites in both posterior femoral muscles, and partly intradermally (267 μg) distributed 3–4 sites in the low dorsal muscles of the mink [15]. In the third immunization the "DNA + protein" group received a boost with 100 μg of recombinant NS1 protein [14], administered subcutaneously as a single injection in a 2% aluminum hydroxide gel adjuvant (Superfos Biosector, Vedbaek, Denmark). One month after the end of the immunization protocol the mink were challenged with 10^5 ID_{50} of type 4 ADV [3,20] administered intraperitoneally. Immediately before each vaccination, mink were anesthetized with 0.1 mL Xylazin 20 mg/mL (Narcoxyl Vet., A/S Rosco, Taastrup, Denmark) and 0.6 mL Ketamin 50 mg/mL (Ketaminol Vet., Intervet Scandinavia AS, Skovlunde, Denmark).

2.4. In vitro protein expression

Plasmid-encoded NS1 expression was assessed by in vitro transfection of pNS1 and pVR1012 into Crandell feline kidney cells. Briefly, 60% confluent cells were transfected using Superfect Transfection Reagent (QIAGEN, Germany) following the instructions provided by the manufacturers. After 4 h at 37 °C and 5% CO_2, cells were washed, incubated at the same conditions for 48 h and harvested to study protein expression by immunofluorescent assay and Western blot.

2.5. Immunofluorescent assay

Cells were fixed in ice cold acetone and incubated for 45 min at 37 °C with a rabbit anti-ADV NS1 polyclonal antibody diluted 1/100 in PBS, 1% Tween 80, 15 mM NaN_3 and 2.5% swine serum. Cells were washed with PBS and incu-