Induction of Immune Responses to Glycoprotein gD of Aujeszky’s Disease Virus with DNA Immunization

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ABSTRACT. In an attempt to produce a DNA vaccine to prevent Aujeszky’s disease, the induction of immune responses against Aujeszky’s disease virus (ADV) gD was investigated in mice. The plasmid was constructed by placing ADV gD gene downstream of murine cytomegalovirus immediate early promoter of expression vector pMYK, which was injected twice on the skin of mice by using a gene-gun. All mice showed neutralizing antibodies against ADV gD at 4 weeks after immunization. The induction of cytotoxic T lymphocytes and splenic natural killer cells was also observed at 6 weeks post immunization. These results indicate that ADV gD gene in the form of DNA vaccine may induce specific as well as non-specific immune responses in vivo.

KEY WORDS: ADV gD, Aujeszky’s disease virus, DNA vaccine.


Aujeszky’s disease virus (ADV) is an alpha herpesvirus that causes a serious infectious disease in swine. This disease is characterized by infection of nasopharyngeal region, which may be followed by central nervous system thereby either death or latent infection [1]. The clinical signs associated with ADV are primarily dependent on the strain of virus, the infectious dose, and more importantly, the age of the swine affected [2]. Aujeszky’s disease has become important in swine industry due to the emergence of virulent virus strains that can kill swine over 3 months old. Attempts to control Aujeszky’s disease in swine have included vaccination with killed or live virus vaccines and passive immunization with hyperimmune serum [9].

In an attempt to make a DNA vaccine to prevent from Aujeszky’s disease, ADV gD gene was chosen as a target molecule with a number of reasons. The glycoproteins of ADV mediate adsorption and penetration of virus and represent a major target for neutralizing antibody [3]. Of the seven genes (gI, gII, gIII, gX, gH, gD, and gp63 [11-13, 16]) coding for glycoproteins, gD is one of the most potent immunogens of ADV infection [10], as such it is a target of neutralizing antibodies that can protect mice and swine from ADV [3, 20]. In addition, vaccination with recombinant gD has been shown to protect animals from ADV [10, 14].

A plasmid was constructed by placing ADV gD gene downstream of murine cytomegalovirus immediate early promoter of an expression vector, and designated pMYK/ADV gD (Fig. 1). To obtain the pMYK/ADV gD plasmid in a large scale, it was transformed into *Escherichia coli* (E. coli) DH5α (Promega) and purified using Qiagen maxiprep kit (Qiagen) according to manufacturer’s protocols. The ratio of OD260 versus OD230, UV scanning pattern, and the pattern of electrophoresis, all showed that the DNA was in a very pure state, and the possible contamination of endotoxin derived from bacteria was negligible (data not shown).

Even though there are a number of methods to deliver DNA vaccine into the body, it has been a general view that the delivery system by using a gene-gun is the most efficient way in inducing immune responses [4]. Therefore we utilized the particle bombardment device to inject plasmid DNA into the skin. The gold coat tubing containing 1.5 µg...
of DNA was shot onto the shaved abdomen of mice. Groups of 5 female C57BL/6 mice (Charles River Laboratories) at 4 weeks of age were injected twice in 2-week intervals with pMYK/ADV gD or pMYK vector without insert as a negative control. The mice in positive control were intramuscularly inoculated twice with half dose of commercial ADV killed vaccine (Daesung Microbiology Research Institute, Korea).

First we examined whether antibodies against ADV gD were produced from immunized animals. To do this, serum samples were taken from tail vein of mice at 2, 4, and 6 week postinoculation and virus neutralization assay was performed as the followings. The serum samples were initially ten-fold diluted and followed by two-fold dilution on a 96 well microplate. The plate was mixed with an equal volume of 2 × 10⁵ TCID₅₀ of ADV, and incubated at 37°C for 1 hr. Viable Vero cells in 50 µl of 10% FBS-MEM were added and incubated at 37°C for 72 hr. Serum neutralizing antibody titers were represented as the reciprocal dilution of serum yielding a 100% reduction in cytopathic effect.

![Fig. 2. The neutralizing antibodies against ADV gD in serum from mice immunized with pMYK/ADV gD. Groups of 5 mice were immunized twice in 2-week intervals with 3 µg of pMYK plasmid (A), and 3 µg of pMYK/ADV gD plasmid (B). The mice in group C were injected with 1/2 dose of ADV killed vaccine (C). Serum neutralizing antibody titers were determined as the reciprocal dilution of serum yielding a 100% reduction in cytopathic effect.](image)

Serum neutralizing antibody titers were represented as the reciprocal dilution of serum yielding a 100% reduction in cytopathic effect. As shown in Fig. 2, the mice immunized with pMYK/ADV gD exhibited neutralizing antibodies at 4 weeks after inoculation, while the mice inoculated with pMYK vector alone did not produce them. The mean neutralizing antibody titers were 30 and 40 for the groups immunized with pMYK/ADV gD and killed vaccine respectively, and almost nothing for the negative control group. The antibody titers at 6 weeks after inoculation were not presented here, because they were not significantly different from those at 4 weeks after inoculation. This result shows that the injection of pMYK/ADV gD into mice without adjuvants efficiently induced humoral immune responses in terms of increased neutralizing antibodies titers, indicating that the antigens expressed in vivo after DNA vaccination probably take a native structure with intact epitopes, and induce neutralizing antibodies. Since we terminated our experiments at 6 weeks after immunization, we could not see if the immune responses after immunization of pMYK/ADV gD lasted long enough thereafter. However, our primary purpose of this study was to see whether pMYK/ADV gD DNA can induce immune responses when injected into the animal, the persistence of immune response was not our current concern.

The protein antigen synthesized in vivo after inoculation in the form of DNA usually are processed in the cytoplasm and efficiently expressed in the groove of major histocompatibility complex (MHC) class I, thereby inducing higher CTL responses [19]. We therefore examined whether CTLs specific for ADV gD were produced after immunization with pMYK/ADV gD. The cytolytic assays were carried out basically according to the method of Slezk and Horan [17] with minor modification; target cells were labeled with PKH-67 green fluorescent dye (Sigma) and after coculturing effector cells and target cells for 6 hr, propidium iodide (PI, Sigma) was added to detect dead cells. To produce a target cell line upon cytotoxic T-lymphocyte (CTL) assays, the pMYK/ADV gD was transfected into B6/wt19 cell line (H-2b) by lipofectin mediated technique [18]. To verify that the transfectants express the authentic ADV gD protein, Western blot analysis of the whole cell lysates with a mAb against ADV gD were performed and a protein with a molecular weight of approximately 55 kD, not present in mock-transfected host cell lysates, was detected, confirming the expression of corresponding ADV gD protein in B6/ wt19 cell line (data not shown).

From the analysis with the Lysis II program of FACStarplus flow cytometer (Becton Dickinson), the cells positive for both PKH and PI could be recognized as dead cells among target cells. The percentage of specific lysis was calculated as follows; % specific lysis = (P-N)/(100-N)× 100, where P is the proportion of dead cells in the presence of effector cells, and N is the proportion of dead cells when only target cells were cultured. The specific killing activity of the splenocytes from mice immunized with pMYK/ADV gD at an effector (E) to target (T) ratio of 30 showed 11.6 ± 1.6% (mean ± standard deviation, n=5), the level of which was higher than that of control mice injected with blank vector (5.6 ± 1.9%) (Fig. 3A). These results clearly indicate that the DNA immunization induces the ADV gD-specific CTL, but it is not sure at present that this level was functionally significant. The additional experiments such as in vitro stimulation of splenocytes with IL-2, which may increase the numbers as well as the activity of T cells before CTL analysis would give rise to clear results about the induction event of CTL after pMYK/ADV gD immunization.

Furthermore we analyzed the induction of NK cells, because one of the distinct advantages in DNA-based immunization has been known to induce the non-specific immune responses like increased NK cells activity. The cytolytic activity of NK cells was examined as did for CTLs, except that NK cell-susceptible Yac-1 cells were used. Figure 3B
shows that splenic NK cells from mice immunized with pMYK/ADV gD exhibited killing activities; at 30 E/T ratio, about 21.8 ± 0.8% specific lysis of target cells was observed. Interestingly, the group of pMYK vector alone also showed significant NK cell activity as compared to control group without prior DNA injection, which showed as less as 7.1 ± 0.3% lysis of target cells at 30 E/T ratio. Recently, a number of previous results with respect to the immune-stimulating properties of plasmid DNA, particularly originated from bacteria, without a cloned gene were reported. The mycobacterial DNA was shown to have an adjuvant effect. A variety of synthetic 45 mer oligonucleotides corresponding to various coding regions of three BCG proteins were reported to induce IFN-γ secretion and augment NK cell activity [21]. The ampicillin resistance gene containing two repeats of the palindromic CpG hexamer 5’-AACGTT-3’ also produced a strong immune response [15]. Since we used pMYK plasmid vector which contains ampicillin resistance gene as well as bacteria-derived genes, the observed NK cell activity would be attributed to those genes on the vector.

In the present study, we demonstrated that ADV gD expressed under control of murine cytomegalovirus promoter induced humoral as well as cellular immune responses specific to ADV in mice, but it remains to be clarified whether these immune responses are sufficient to protection from the challenge experiment in mice with ADV, as did Ho et al. [8]. Recently similar studies regarding the development of DNA vaccines to prevent pigs from ADV infection were reported [5–7]. When the DNA plasmid was injected into the swine and challenged with relevant ADV, protective immunity was established. In this regard, it is of particular interest to study whether the pMYK/ADV gD has potential to confer protective immunity to swine against ADV infection.

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