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A New Strategy in the Immunization of Dna Vaccine Against Hog Cholera Virus

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Abstracts: Swine fever is caused by the hog cholera virus (HCV), and is an important source of pathogen-related economic loss within the pig industry worldwide. Control of this disease is mainly by vaccination and commercial vaccines are not totally effective. DNA vaccine have many advantages over conventional vaccines, but handicap is its low efficacy .In this study, a new formulation of DNA vaccine was developed to improve its efficacy, results showed this new vaccine can enhance both humoral and cellular immune response compared with naked DNA vaccine.

Key words: Hog cholera virus, DNA vaccine, Immunization

Introduction

Direct injection of plasmid DNA has been demonstrated potential as a new vaccine strategy. The first peer-reviewed report of protective immunity and cytotoxic T lymphocyte (CTL) induction in mice after intramuscularly injection (i.m) of a naked DNA plasmid appeared in (Wolff *et al.*, 1990), subsequently, the use of DNA vaccines in pre-clinical studies has become well established (David and McCluski, 1999; Strugnell *et al.*, 1997; Kalinna, 1997), with report of protective immunity in many different independent studies. In recent studies (Ugen *et al.*, 1998), 1-2mg of DNA was inoculated on multiple sites of animal, both antibody and CTL responses were induced in non-human primates, however, the use of very high doses of DNA is not good for practical application, therefore, there is a clear requirement to find a method that can induce immunity with lower dosage of DNA.

There are lots of strategies that have the potential to improve the efficacy of DNA vaccine (Feltquate, 1998), including modification of expressing vector, improvements in DNA delivery or the inclusion of adjutants. The monophony lipid A has been reported (Stanberry *et al.*, 2002) could enhance both humoral and cell-mediated immune responses to DNA vaccination against human immunodeficiency virus type 1. DNA vaccine (Kashala *et al.*, 2002) formulated with QS saponin adjuvant via intramuscularly and intranasal routes could also induce systemic and mucosal immune responses. Manmohan S (Singh *et al.*, 1998) had developed a potent delivery system for DNA vaccines, the cationic micro particles. GM-CSF (Somasundaram *et al.*, 1999) also plays an important role in the immunization of DNA vaccine. It is reported (Millan *et al.*, 1998) that bacterial DNA-sequences called immunostimulatory sequences can be potent adjuvant. Non-methylated, palindrome DNA-sequences containing cpG-oligodinucleotides (CpG-ODN) can activate an "innate" immune response by activating monocytes, Nk cells, dendritic cells and B cells in an antigen-independent manner. Indeed, methylation of the CpG-ODN reportedly abrogates the immunogenicity of the DNA vaccine. Thus the use of large amounts of plasmid for immunization may not only overcome the low transfection efficiency in vivo, but may also serve as an adjuvant, driving a Th1-type response.

We have secured a highly immunogenic HCV E2 gene 1.25 constructs as well as 2.5 constructs previously, preliminary immunogenicity studies with these constructs in pigs had shown naked DNA only provided a protection of 57%, In order to improve the efficacy, a new formulation of this DNA vaccine (pHCV2.5) was developed and tested in rabbit, using crude bacteria instead of purified DNA.

Materials and Methods

Animals. Female rabbit (1-2kg) were purchased from animal center of Hong Kong University.

Cells, plasmid and Virus. PK15 obtained from ATCC was used in neutralization assay, HCV commercial vaccine was bought from China (Guangzhou). pcDNA 3.1 was brought from Invitrogen. Co.

Preparation of DNA vaccine. The recombinant plasmid was prepared as follows; briefly, pcDNA3.1 was used as the cloning vector.

PHCV2.5 contains three consecutive genes of HCV, E0, E1 and E2, which were isolated from a HK regional strain of HCV with a RT-PCR; the insert size was about 2.5kb. The formulated DNA vaccine was prepared by the following protocol, inoculate 1ml bacteria seeds which is propagated from a single colony to 1.5L LB broth with 1mg/ml Ampicillin, incubate at 37°C and shaking at 200rpm overnight, spin down and dry the bacteria pellet, weighing and reconstitute with PBS to a given concentration (o.1ml contains 100ug DNA), sonication for 10 min, and add 1mg/ml gentamycin, put it at 4°C for 30min, then it is ready for administration.

Experimental protocol: 5 rabbit was used in this study; each of them was housed in a separate unit. Rabbit NO.1 and NO.2 was injected pHCV1.25 DNA vaccine o.1ml and o.5ml respectively, Rabbit NO.3 and NO.4 was injected purified pHCV1.25 and commercial vaccine respectively, NO.5 set as control. Booster at 7days interval for 2 times. Administrated by two methods, Blood samples were collected from each rabbit at a 10 days interval, two kinds of blood were collected at the same time, one is coagulated blood, and another is non-coagulated blood, used for humoral immune response and cellular immune response analysis respectively. An ELISA kit was used in this study (IDEXX Co.),

Flow cytometry assay: Briefly, Preparation of single cell suspensions (Peripheral blood lymphocytes). Whole blood were collected into heparinized syringes and placed on ice, the blood was diluted in Hank's balanced salt solution (HBSS) without phenol red plus 1.5% fetal bovine serum (Sigma Co.) and sieved through a 15ml rayon wool column primed with HBSS. 5 ml of the filtered blood was laid over 5ml Ficoll-Hypaque (Sigma) and centrifuged at 2000rpm for 10min. The interface buffy coat was removed with a Pasteur pipette and rinsed three times with 5ml HBSS. After final rinse, total lymphocyte from 10^4 cells per sample was determined in a flow cytometeric apparatus.

Virus neutralization assay: HCV neutralizing antibodies (VNAb) were titrated by the rapid fluorescent focus inhibition test (RFFIT). Anti-HCV VNAb titers are expressed in serum dilution. Using HCV C strain as the reference or as the reciprocal serum dilution (rd) that inhibited 50% of the fluorescent focus.

Histological examination: At the end of experiment, muscles of rabbit was resected and fixed with 10% buffered formalin and were embedded in paraffin. Sections were used for the observation of microscopic lesions.

Results

Serum antibody response of formulated DNA vaccine in rabbit (Fig.1): When rabbit was inoculated with the formulated DNA vaccine, either low dose or high dose both had a humoral response two weeks later, as it checked by ELISA and neutralization assay, the VNAb titer continued to increase after booster, Compared with purified plasmid DNA vaccine and commercial vaccine, the response of neutralization antibody in the rabbit inoculated with the formulated DNA vaccine was better than them.

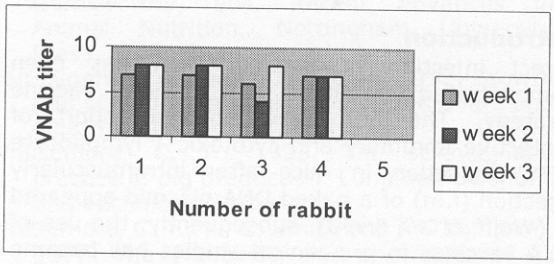

Fig. 1: Neutralization titer of rabbit serum after injected with a formulated DNA vaccine

Cellular immune response: The un-clotted blood of the rabbit was checked by using a flow cytometric assay, mophocyte, monocyte and granular cell of the peripheral blood was counted respectively. Results showed rabbit immunized with the formulated DNA vaccine had a high concentration of inflammatory cells, especially the granular cell, high dose inoculated rabbit ranked the first.

Microscopic lesions in DNA vaccine injected muscles: DNA vaccine was injected in the right biceps femurs muscle of rabbit, microscopic examination showed massive accumulation of monocyte, reflected strong inflammation was induced, whereas rabbit inoculate with the commercial vaccine and purified DNA vaccine only had a mild infiltration of inflammatory cell.

Discussion

DNA vaccination is a promising technology to prevent diseases outbreak in farm and companion animals, efforts must now be concentrated on improving immunity in a specified animal by searching for appropriate

adjutants (CpG and other immunostimulation molecules) and the optimal route of administration, targeting the expression plasmids. The cost of the vaccine must also be taken into account; plasmid purification is expensive and amounts injected need to be adjusted.

Based on this idea, we try to find a convenient and cheapest way for DNA vaccination.

We have provided the first evidence that this formulated DNA vaccine had a better efficacy than purified DNA vaccine, it can induce both humoral and cellular immune response. The higher inoculation dose, the better immune response, the bacteria protein may act as an adjuvant in this case. The detailed mechanism need to be further studied.

Since this formulated DNA vaccine didn't cause fever or death of rabbit, it was suggested that rabbit may have endurance to the toxin of bacteria, of course, the long time treatment to the bacteria by ultrasonic may destroy part of the toxin. There was almost no gross lesion in the injection site, the rabbit injected with a low dose had been well absorbed, only the rabbit injected with the high dose had some tyromatosis, whereas histological examination found massive infiltration of inflammatory cells, it reflected strong inflammation had occurred.

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References

Wolff, J.A., R. W. Malone, P. Williams, W. Chong, G. Acsadi, A. Jani and P. L. Felgner, 1990. Direct gene transfer into mouse muscle in vivo. Science: 247, 1465-1468.

Davis, H.L. and M. J. McCluskie, 1999 DNA vaccines for viral diseases. Microbes Infect., 1: 7-21.

Strugnell, R.A., D. Drew, J. Mercieca, S. Dinatale, N. Firez, S. J. Dunstan, C. P. Simmons and J. Vadolas, 1997. DNA vaccines for bacterial infections. Immunol. Cell Biol., 75: 364-369.

Kalinna, B.H., 1997. DNA vaccines for parasitic infections. Immunol. Cell Biol., 75, 370-375.

Ugen *et al.*, 1998

Ugen, K.E., S. B. Nyland, J. D. Boyer, C. Vidal, L. Lera, S. Rasheid, M. Chattergoon, M. L. Bagarazzi, R. Ciccarelli, T. Higgins, Y. Baine, R. Ginsberg, R. R. Macgregor and D. B. Weiner, 1998. DNA vaccination with HIV-1 expressing constructs elicits immune responses in humans. Vaccine 16: 1818-1821.

Feltquate, D.M., 1998. DNA vaccines: Vector design, delivery, and antigen presentation. J. Cell. Biochem., S30/31: 304-311.

Stanberry, L.R., S. L. Spruance, A. L. Cunningham, D. I. Bernstein, A. Mindel, S. Sacks, S. Tyring, F. Y. Aoki, M. Slaoui, M. Denis, P. Vandepapeliere, G. Dubin, 2002. Glycoprotein-D-adjuvant vaccine to prevent genital herpes.N. Engl. J. Med., 347:1652-61

Kashala, O., R. Amador, M. V. Valero, A. Moreno, A. Barbosa, B. Nickel, C. A. Daubenberger, F. Guzman, G. Pluschke, M. E. Patarroyo, 2002. Safety, tolerability and immunogenicity of new formulations of the Plasmodium falciparum malaria peptide vaccine SPf66 combined with the immunological adjuvant QS-21. Vaccine, 20:2263-77

Singh, M., J. R. Carlson, M. Briones, M. Ugozzoli, J. Kazzaz, J. Barackman, G. Ott, D. O'Hagan, 1998. A comparison of biodegradable microparticles and MF59 as systemic adjuvants for recombinant gD from HSV-2.Vaccine, 16: 1822-7

Somasundaram, C., H. Takamatsu, C. Andréoni, J. -C. Audonnet, L. Fisher, F. Lefèvre and B. Charley, 1999. Enhanced protective response and immuno-adjuvant effects of porcine GM-CSF on DNA vaccination of pigs against Aujeszky's disease virus. Vet. Immunol. Immunopathol., 70: 277-287.

Brazolot Millan, C.L., R. Weeratna, A. M. Krieg, C. A. Siegrist and H. L. Davis, 1998. CpG DNA can induce strong Th1 humoral and cell-mediated immune responses against hepatitis B surface antigen in young mice. Proc. Natl Acad. Sci. U. S. A. 95: 15553-15558.