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Advances in Studies on Vaccines of Foot-and-mouth Disease

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ABSTRACT

The Foot-and-mouth Disease (FMD), which is the most devastating disease of livestock husbandry, drastically hinders the development of stock raising. At present the main FMD vaccine is still traditional inactivated vaccine, as its good performance in protective activity and immune efficacy, it is widely used in the world for preventing and controlling this disease, especially in developing countries. However, people have realized many disadvantages of inactivated vaccine. In order to resolve these problems, the scientists are conducting some new research about novel FMD vaccine to seek for more secure and effective vaccines. Nowadays, with the rapid development of molecular biology, researchers are paying high emphasis on novel FMD vaccines, such as, synthetic peptide vaccine, epitope-based vaccine, DNA vaccine, chimeric vaccine and so on. There is no doubt that these new-type FMD vaccines will make great contribution to the prevention and control of FMD. This study reviewed the international research progress on the traditional vaccines and novel vaccines of FMD.

Key words: Foot-and-mouth disease, new-type vaccines, conventional vaccines, epitope, synthetic peptide

INTRODUCTION

Foot-and-mouth Disease (FMD) is an acute, highly contagious disease of cloven-hoofed animals and is endemic in many regions in Africa, Asia and South America (Cottam *et al.*, 2008; Schumann *et al.*, 2008; Shawky and Daoud, 2005; Ahad *et al.*, 2002). It is the most devastating disease of livestock husbandry (James and Rushton, 2002), has caused huge loss economically and drastically hinders the development of stock raising. Economic loss in Bangladesh because of calf mortality, milk yield reduction resulted from Foot-and-mouth Disease Virus (FMDV) infection would stand at US\$ 163329 per year (Howlader *et al.*, 2004).

It is caused by the Foot-and-mouth Disease Virus (FMDV), a member of the genus *Aphthovirus* of the family Picornaviridae, which is highly transmissible and can causes high morbidity outbreaks with moderate to low mortality in most cases. When a host is infected by FMDV, the host protein will be subverted and its function will also be altered to aid in virus replication

(Chase and Semler, 2012). The disease occurs frequently during the winter season (Verma *et al.*, 2008). In the mountainous area of Turkey, the FMD incidence in goat is 23% (Darcan *et al.*, 2005).

There are seven serotypes of FMDV (A, O, C, Asia1, SAT1, SAT2 and SAT3) with multiple subtypes within each serotype (Paprocka, 2006). The harmfulness of FMD is conspicuous (Sumption *et al.*, 2008; Gibbs, 2003; Sobrino *et al.*, 2001) and there is no effective way to control this disaster (Mort *et al.*, 2005). FMD is the most important animal disease limiting trade of animals and animal products (Rufael *et al.*, 2008; Barasa *et al.*, 2008; Perry and Rich, 2007). In spite of some clinical illness caused by FMDV infection such as mastitis are curable (Sharma, 2008), almost no effective drugs are currently available to cure this disease. Although vaccination is an effective way to limiting the spread of FMDV (Rizk *et al.*, 2011), it is unlikely that a single vaccine approach will solve the many shortcomings of current vaccines (Rodriguez and Grubman, 2009). In this manuscript, based on the researches in our lab, we reviewed the international research progress on FMD vaccines. We hope it is helpful to the FMD vaccines researchers.

FMD VACCINES

FMD is devastating that it has a significant impact on world economies and prevails in many countries and areas throughout the world (James and Rushton, 2002). People have realized vaccination is an effective measure to prevent and control FMD (Rodriguez and Gay, 2011). The current vaccines include conventional vaccines and novel vaccines, the conventional vaccines include attenuated vaccine and inactivated vaccine. And the new-type vaccines include recombinant protein and peptide vaccines, empty capsid vaccines, live attenuated vaccines and so on (Rodriguez and Grubman, 2009). They, respectively play different roles in the prevention and control of FMD in different epidemic areas and stages.

At present the main FMD vaccine is still traditional inactivated vaccine, as its good performance in protective activity and immune efficacy, it is widely used in the world for preventing and controlling this disease, especially in developing countries (Rodriguez and Gay, 2011). However, people have realized many disadvantages of traditional vaccine, such as, virulence recurrent, incomplete inactivation and even virus leak (Rodriguez and Gay, 2011). So many researchers have attempted to develop more effective and secure vaccines that may provide additional tools to help control FMD in the future.

Inactivated vaccine: Conventional inactivated vaccines were produced by these field virus strains which were disposed by inactivators and adjuvants after a series of amplifications. In 1931, Society for microbiology held for the first FMD academic conference in Berlin and laid the foundation for FMD vaccine research. In 1990, an international bank, which is based on concentrated FMDV preparations stored in the gaseous phase of liquid nitrogen, has been established at the Pirbright Laboratory of the AFRC Institute for Animal Health (Doel and Pullen, 1990). Frenkel (1951) described the procedures and facilities for mass production of FMDV vaccine. Some researchers inactivated FMDV by virion-associated endonuclease, the results showed endonuclease is equal to or better than formaldehyde or ethyleneimine in guinea-pig potency tests (Amadori *et al.*, 1987).

Attenuated vaccine: Attenuated vaccine played a key role in controlling the FMD pandemic in Europe, however, it was discontinued because of various reasons. On the one hand, the weak phenotypes of these attenuated viruses were not always stable and the weak features were host

specific, that is to say, one attenuated virus may showed its weak features to a certain animal. Further more, it may not able to stimulate inoculated animals to produce effective protective immune response as its excessive weakening. On the other hand, although FMD NSP ELISA was used to distinguish native infection from vaccinated (Raof *et al.*, 2011; Hassanein *et al.*, 2011), the animals which inoculated attenuated vaccine could also produce non-structure protein, so it is difficult to distinguish natural infection and vacation.

Genetic engineering live vector vaccine: Live vector vaccine is a kind of recombinant protein expressed by animals inoculated with such recombinant virus whose genome contains FMDV immunogenic genome and nonpathogenic virus vector genome. Live vector vaccine is more effective than inactivated vaccine because the inoculated animals can not only produce antibody against FMDV but also virus vector (Balamurugan *et al.*, 2004). Many vectors have been developed, for instance, recombinant vaccinia virus (Berinstein *et al.*, 2000), recombinant fowlpox virus (Zheng *et al.*, 2006), recombinant pseudorabies virus (He *et al.*, 2008), recombinant infectious bovine rhinotracheitis virus (Kit *et al.*, 1991) and so on.

DNA vaccine: DNA vaccine is a kind of expression plasmid, containing virus antigen gene, which could stimulate the vaccinated animals to produce a protective immune response (Rawat *et al.*, 2007; Liu, 2006). The animal has the ability of absorbing naked DNA molecules. And we could treat animals by transfecting target clone gene directly to them. Wang *et al.* (2011) reviewed the approaches to improve DNA vaccine efficacy and they demonstrated that improved target clone gene can not only increase the protective effects but also reduce vaccination doses and toxicity. In recent years, DNA vaccine is widely used to control FMD. Such as Benvenistia *et al.* (2001) used a FMDV expression constructs to protect pigs from FMDV infection. In addition Wang *et al.* (2008) found co-inoculating IL-15 construct with pcD-VP1 (FMDV DNA vaccine) to animals could enhance the Cell-mediated Immunity (CMI) compared to the pcD-VP1 alone.

Transgenic plant vaccines: Antigen gene was introduced into plants by the use of transgenic technology and then we could gain the transgenic plants which are able to express relevant protein. The extracts of transformed plant materials could induce immunological response (Aliahmadi *et al.*, 2006). FMD transgenic plants vaccines was one of early and successful case. The use of transgenic plants as a biological reactor to expressed recombinant protein vaccine is a very attractive and cheap production system, it may replace the traditional high costing vaccine production system someday. Mice immunized with the leaf extracts of transformed plants which could express VP1 of FMDV, all mice were protected against FMDV challenge (Carrillo *et al.*, 1998). And this is the first study showing protection against a viral disease by immunization with an antigen expressed in a transgenic plant. Wigdorovitz *et al.* (1999) immunized mice with the leaf extracts of transgenic alfalfa which could express the structural protein VP1 of FMDV and the immunized mice were protected against FMDV challenge. A transgenic alfalfa containing the genes encoding the polyprotein P1 and the protease 3C of FMDV was constructed by Dus Santos *et al.* (2005) and the immunized mice were completely protected when challenged with the virulent virus. Pan *et al.* (2006) used the VP1 production expressed by transformed solanum tuberosum to immune guinea pigs, it indicated that the expression product had immunogenicity and could effectively induce the specific antibodies against FMDV. Two years later, Pan *et al.* (2008) fond the production of transgenic tomato plants that express the structural polyprotein, P1-2A and protease, 3C of FMDV could also fully protect pigs from FMDV challenge.

Protein vector vaccine: FMD protein vector vaccine is some certain fusion protein made up with B\T cell epitope and macromolecular proteins or virus-like particles. This kind of vaccine could enhance cellular immune response as the antigen presentation of protein vehicle or virus-like particles. Many vectors have been developed, such as, KLH, BSA, MSA, MIM, MWS, AHJ, SWS, the heavy chain of IgG and some molecular chaperones. Pfaff *et al.* (1982) connected VP1 144-159aa of FMDV to BSA, KLH and cow parathyroid globulin and used them to inoculate rabbit, the results showed BSA is better than the other two. Guinea pigs inoculated with KLH-VP1 141-160aa could fully resist a challenge infection (Bittle *et al.*, 1982). Zamorano *et al.* (1995) used BSA-VP1 135-160aa to inoculate mice, the mice could effectively induce the specific antibodies against FMDV.

Empty capsid vaccine: Empty capsid vaccine is a kind of new-style vaccine, which contains all antigenic epitopes while lacks of virus nucleic acid (Rodriguez and Grubman, 2009; Rowlands *et al.*, 1975). As this kind of vaccine can not produce non-structural protein, so we can distinguish vacation from native infection (Fu *et al.*, 2011). And this vaccine is more secure because of lacking nucleic acid. To some extent, empty capsid vaccine is live vector vaccine, however, if we use the extract of expression vector system to inoculate animal it is different from live vector vaccine. A recombinant silkworm *Baculovirus* Bm-P12A3C constructed by Li *et al.* (2008), which contain the intact P1-2A and 3C protease coding regions of FMDV Asia 1/HNK/CHA/05, could protect four out of five vaccination animals from homologous FMDV challenge.

Chimeric viral vaccines: With the help of reverse genetics technique the antigen coding areas of a known vaccine strain has been substituted with that from another serotype or epidemic isolates. Thus we gain a recombinant, called chimeric virus. And chimeric viral vaccine is made from this kind of new virus. Chimeric viral vaccine develops more quickly than traditional vaccine. A chimeric FMDV vaccines, in which the VP1 G-H loop had been substituted with that from another serotype, could fully protect cattle from challenge 21 days post-vaccination (Fowler *et al.*, 2008). A cross-serotype chimeric virus engineered by Blignaut *et al.* (2011), could induce pigs to produce neutralizing antibodies and protect vaccination pigs against homologous FMDV challenge.

Epitope peptide vaccine: Epitope peptide vaccine is made from antigens amino acid sequence through artificial chemical synthesis or genetic engineering expression. At present, the key study of FMD epitope peptide vaccine lays on expression of antigens gene or adjuvant gene. OmpA fusion proteins inserted with the VP1 sequence of FMDV could elicit virus-specific immune responses in rabbits (Ruppert *et al.*, 1994).

Synthetic peptide vaccine: Peptide vaccine is chemically synthetic peptides contain a number of B cell and T cell antigenic epitopes. The main difference between epitope peptide vaccine and synthetic peptide vaccine is that the epitope number of epitope peptide vaccine is larger than that of synthetic peptide vaccine. On the one hand, synthetic peptide vaccine costs too much and the technology of protein synthesis is limited. On the other hand, epitope peptide vaccine could add many other epitopes at the help of vector. Both of the two vaccines do not exist the problem of virulence recurrent or inactivated incompletely. They have great advantages in the control of FMD. Wang *et al.* (2002) designed a FMDV peptide-based vaccine for swine. The peptide immunogen has the VP1 G-H loop domain and a novel promiscuous T helper site. 20 out of 21 immunised pigs were

protected from infectious challenge by FMDV O1 Taiwan. Rodriguez *et al.* (2003) designed a synthetic consensus peptide comprising the VP1 G-H loop region of FMDV type-O and a non-VP1 T-helper epitope. Although the synthetic peptide induced an antibody response in cattle, it failed to confer protection against FMDV challenge. Wiesmuller constructed a novel synthetic peptide vaccine P3CSS-FMDV-VP1 (135-154). The vaccine could induce a long-lasting high protection against FMDV and serotype-specific virus-neutralizing antibodies in guinea-pigs (Wiesmuller *et al.*, 1989).

THE STUDY ON ANTIGENIC FMDV EPITOPES

As mentioned above, synthetic peptide vaccines, live vector vaccines, epitope peptide vaccines contain the antigenic epitopes, so the study on epitopes is crucial for the development of these novel vaccine. So far, many epitopes have been discovered, such as, VP1 144-159aa (Pfaff *et al.*, 1982); VP1 25-41aa, 200-213aa (Bittle *et al.*, 1982); VP4 20-35aa (Van Lierop *et al.*, 1995; Blanco *et al.*, 2000); 3A 11-25aa, 3A 21-35aa, 3C 166-180aa (Blanco *et al.*, 2001); 3D 51-65aa, 3D 91-115aa, 3D 181-200aa, 3D 341-360aa, 3D 381-400aa (Garcia-Briones *et al.*, 2004); 3D 301-315aa, 3D 326-340aa, 3D 346-360aa, 3D 351-365aa, 3D 356-370aa, 3D 406-420aa, 1A 62-76aa, 1A 67-81aa (Gerner *et al.*, 2006); 3D 16-33aa (Yang *et al.*, 2007); VP1 66-80aa (Gerner *et al.*, 2007); VP1 106-115aa, 4-13aa (Liu *et al.*, 2011); VP1 1-12aa, 17-29aa, 194-211aa, VP2 40-50aa, VP3 26-39aa, VP4 30-41aa (Zhang *et al.*, 2010) and so on.

There is no doubt that the study on epitopes will greatly promote the development of novel vaccines. Thanks to the consecutive discovery of new epitopes, we could produce poly-vaccine containing epitopes of different serotypes (Hong *et al.*, 2007) to overcome the MHC restriction more easily. It is obviously that epitope plays a key role in exploiting new-type FMDV vaccines.

CONCLUSION

In conclusion, at the moment the main FMD vaccine is still traditional inactivated vaccine, especially in developing countries. In recent years, as the genome sequencing technology and protein peptide chemical synthesis technology become mature, especially the proposal of epitopic biology, more and more FMDV antigenic epitopes are revealed. Researchers take an interest in synthetic peptide vaccines in order to develop more effective and secure vaccines that may help control FMD in the future. As the production of multivalent vaccine is difficult due to the high diversity and variability of pathogenic microbes, people gradually pay more attention to polypeptide vaccine. The selected epitope sequences are highly conserved in different serotypes and this is the key guarantee of cross immune response of vaccine. The principle of synthetic peptide vaccine is based on the amino acid sequence which is an unique design idea that represents a new design direction of vaccine in recent years. Compared with traditional vaccine, synthetic peptide vaccine has many advantages: (1) it can be recognized and combined by many MHC of different genetic background, so it can be presented efficiently (2) it has unique superiority on cell-mediated immune (3) it is safer than attenuated vaccine and inactivated vaccine and (4) native infection can be distinguished by designing non-structural protein antigenic epitopes in chemical synthetic peptides.

Among the FMD new-style vaccines, most of them have intersection, such as, synthetic peptide vaccines, epitope peptide vaccine, protein vector vaccine are all based on antigenic epitopes; for another, DNA vaccine and live vector vaccine are both based on gene expression. Different kinds of vaccines play different role in different areas as well as in different epidemic stages. They are tuneable and coadjuvant in the prevention of FMD.

A secure and effective vaccine should be able to expedite eradication through vaccination and could also minimize the need for control by culling. There is no doubt that the new-type vaccines will make great contribution to the prevention and control of FMD.

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