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Review

Foot and mouth disease virus vaccines

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ABSTRACT

Foot and mouth disease (FMD) is a highly infectious and economically devastating disease of livestock. Although vaccines, available since the early 1900s, have been instrumental in eradicating FMD from parts of the world, the disease still affects millions of animals around the globe and remains the main sanitary barrier to the commerce of animals and animal products. Currently available inactivated antigen vaccines applied intramuscularly to individual animals, confer serotype and subtype specific protection in 1–2 weeks but fail to induce long-term protective immunity. Among the limitations of this vaccine are potential virus escape from the production facility, short shelf life of formulated product, short duration of immunity and requirement of dozens of antigens to address viral antigenic diversity. Here we review novel vaccine approaches that address some of these limitations. Basic research and the combination of reliable animal inoculation models, reverse genetics and computational biology tools will allow the rational design of safe and effective FMD vaccines. These vaccines should address not only the needs of FMD-free countries but also allow the progressive global control and eradication of this devastating disease.

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1. Introduction

The last decade has seen a renewed public and political interest on foot and mouth disease (FMD) due to its potential as a bio-terrorist threat and some high-profile disease incursions in previously FMD-free countries in Asia, Europe and South America [1,2]. Control methods varied among these regions but the ultimate result was the demise of millions of animals, the loss of billions of US$ in various economic activities including tourism, agriculture and trade and serious social impacts [3]. The public and politicians alike ask why if effective vaccines are available they are not always used for outbreak control? Why countries using vaccines to control FMD are penalized with animal trade restrictions? The answers to these questions are explained at least in part by critical shortfalls of current inactivated antigen vaccines and lack of clear understanding of disease transmission and pathogenesis. This review will update previous reviews on FMD vaccines [4] highlighting the limitations of current vaccines and providing some perspectives toward upcoming molecular vaccines.

2. FMDV overview

FMD is perhaps the most important animal disease limiting trade of animals and animal products [5–7]. FMDV is highly trans-

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missible and causes high morbidity outbreaks with moderate to low mortality in most cases. Hallmarks of FMD virus (FMDV) infection include the appearance of vesicular lesions in epithelia of the mouth and coronary bands of the hoof, the highly contagious nature and the multiple virus antigenic types and subtypes [8].

After aerosol exposure of cattle FMDV first replicates in the pharynx. In 24–48 h the virus invades the blood stream and shortly thereafter lesions appear in the mouth and feet of susceptible animals [9]. Viremia usually disappears after 3–4 days but virus replicates to very high titers (>8 log 10 infectious units per ml) at lesions sites and is shed in the air and body fluids. Between 5 and 10 days after their appearance, lesions resolve and virus is no longer found at the lesion sites and can only be recovered from pharyngeal fluid and tissues [9]. The virus establishes persistence in the pharyngeal region of approximately half of the infected animals, even vaccinated animals protected from clinical disease, that become long-term carriers [10,11]. Although the role of persistence in virus transmission remains unclear the fact that vaccines fail to prevent the carrier state is arguably one of the reasons vaccination is not the first choice for outbreak control in non-enzootic countries, since infected countries need to demonstrate freedom of viral infection before they regain FMDV-free status.

The current global status of FMD distribution shows geographic areas where viral activity persists over long periods of time in the local population [1]. Most of these "hot spots" of viral activity are located in poor countries that lack the adequate veterinary services and resources necessary to undertake control and eradication efforts. Trade restrictions of animals and animal products have failed to prevent incursions of FMDV from enzootic hot spots into FMD free areas and hemispheric eradication efforts have met limited success [12,13]. Inactivated FMD vaccines have proven to be an important component of control and eradication strategies both in enzootic and non-enzootic settings. However, hot spots of viral activity remain resistant to control efforts and viral incursions into non-enzootic regions are often controlled by mass slaughter of susceptible animals without the use of vaccines [13]. Here we review the advantages and shortcomings of current inactivated vaccines and novel molecular vaccines that may provide answers to some of the problems faced by the efforts to control and eradicate FMD.

3. FMD inactivated antigen vaccines

3.1. Brief history

FMDV was the first animal virus described as an etiological agent by Loeffler and Frosch in 1897 and FMD vaccines were among the first animal vaccines to be developed, with efforts to immunize animals by exposure to infectious virus beginning at the end of the 19th century [14]. But a practical vaccine was never realized due to the unpredictability of viral virulence and the existence of multiple viral serotypes (Vallée, 1922). The first inactivated vaccine was developed by Waldmann et al. around 1937 using vesicular fluid obtained from tongues of deliberately infected cattle, and subsequently inactivated with formaldehyde [15]. But industrial production of inactivated vaccines did not begin until the 1950s after Frenkel described the culture of tongue epithelium from healthy slaughtered animals [14]. Further breakthroughs in inactivated FMD vaccine production included the growth of FMDV in BHK cell suspension cultures in the 1960s [16], the introduction of ethylene imines for FMDV antigen inactivation [17,18], and the use of oil-adjuvants in the 1970s [19]. For extensive reviews on the history of FMD vaccines see Lombard et al. [14] and Doel [20].

3.2. Modern vaccine production and antigen purification

As inactivated vaccines evolved, several problems manifested themselves, such as the incomplete viral inactivation of formaldehyde treated antigens. This problem was solved by the introduction of BEI inactivated antigens. In early vaccine production systems concentration of the antigen was achieved through the use of aluminum hydroxide gel adsorption, or polyethylene glycol precipitation. Although these methods are effective and still in use in some parts of the world, they have been largely replaced by industrial ultra-filtration and chromatography in order to remove unwanted cellular protein contaminants and viral non-structural proteins. The need to further purify vaccine antigens arose not only to prevent unwanted allergic reactions to cell proteins in animals after multiple vaccinations but also to allow differentiation of infected from vaccinated animals during control campaigns [20].

At the beginning of the 21st century the protocol for production of inactivated FMD vaccines allows the use of serological tests that can differentiate infected from vaccinated animals, formulation of vaccines that include multiple serotypes and subtypes and a number of adjuvants [20]. However, the basic technology for vaccine production has remained the same, still requiring the growth of large volumes of virulent FMDV, subsequent virus inactivation and antigen concentration and purification. This raises concerns with biosafety issues and causes countries like the United States to prohibit vaccine production on its mainland. Additionally, FMD vaccines like other killed antigens do not induce broadly reactive long-term protection, require multiple vaccinations to maintain good levels of herd immunity and require periodic inclusion of new viral strains into the vaccine formulation to cover new viral subtypes against which existing vaccines no longer protect. Other important shortcomings of current inactivated vaccines include short shelf life, the need for adequate cold chain of formulated vaccines, and difficulties of certain serotypes and subtypes to grow well in cell culture for vaccine production. In order to address these problems basic research and new technologies are necessary.

4. Novel molecular vaccines

4.1. Recombinant protein and peptide vaccines

By the mid-1970s researchers had developed information concerning the virus capsid structure and determined that one of the capsid proteins, VP1, had a prominent surface exposure [21,22]. Based on this information a number of strategies were utilized to develop protein vaccines as alternatives to the inactivated vaccine. VP1 was isolated from purified virus and induced a neutralizing antibody response in swine [23] and protected both swine and cattle against FMDV challenge [24]. Utilizing recombinant DNA technology it was shown that VP1 produced in *E. coli* protected both swine and cattle from virus challenge [25]. Additional studies revealed that portions of VP1 could induce a neutralizing antibody response and nucleic acid sequencing of different strains of the virus showed variation in these protein regions. These regions represented the variable G–H loop found on the surface of the FMDV capsid [26] and the carboxy-terminal region of VP1 and corresponded to B cell epitopes. Bittle et al. [27] synthesized peptides corresponding to these regions and demonstrated that the peptides could induce high levels of neutralizing antibody in cattle and protected guinea pigs from challenge. Subsequently DiMarchi et al. [28] immunized cattle with peptides representing these regions of VP1 and demonstrated protection of 2 of 3 animals. Additional studies combining these peptides with peptides that represented FMDV T-cell epitopes showed that the T-cell peptides were recognized by a significant number of cattle and pigs. Various groups have used

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other systems to produce B and T-cell peptides including transgenic plants, or plants infected with recombinant viruses as well as other methods of antigen delivery such as viral vectors and naked DNA [29–33]. In a large-scale evaluation of peptide vaccines in cattle Taboga et al. [34] found that peptides administered in several doses and vaccination schedules afforded, at best, 40% protection and that virus escape mutants contained amino acid substitutions at the major antigenic sites represented in the peptide vaccines. Similar results were obtained by Rodriguez et al. [35].

The results of these studies suggest that peptide vaccines representing only a limited number of antigenic sites and/or T-cell epitopes of the virus are not able to induce significant protection. Furthermore, the above sites represent only continuous regions of the virus capsid. However, some of the antigenic sites on the virus are discontinuous and involve different regions of a capsid protein or more than one protein. In addition, the quasispecies nature of FMDV [36] invites the selection of antigenic variants that could cause outbreaks in animals vaccinated with peptide vaccines.

4.2. Empty capsid vaccines

An alternate approach to protein or peptide vaccines is the development of immunogens that contain the entire repertoire of immunogenic sites present on the intact virus particle thereby decreasing the possible selection of antigenic variants from the quasispecies. Empty viral capsids are virus particles lacking nucleic acid which are naturally produced in infected cells and are as immunogenic as virions [37,38]. A number of groups constructed plasmids containing the capsid and the 3C protease ($3C^{pro}$) coding regions [39–41]. The latter gene product is required for processing the capsid precursor protein into the structural proteins VP0, VP3 and VP1 [42]. Utilizing various expression systems empty capsids were synthesized and assembled in cell culture.

Subsequently a number of approaches have been used to deliver the products either as proteins expressed in *E. coli* [43] or recombinant baculoviruses [44] or by direct intramuscular inoculation of recombinant vectors including naked DNA [45], poxviruses [46,47], and human adenovirus [48–50]. Thus far the most successful strategy has been the delivery of the FMDV capsid sequence with a recombinant, replication-defective human adenovirus type 5 (Ad5). This vector has the capacity to incorporate 5–8 kbp of foreign DNA, can only grow in complementary cells expressing the deleted Ad5 genes adding to the level of safety, but can infect multiple animal species including cattle and swine. An additional advantage of direct inoculation of a live vector is that the FMDV capsids are expressed and assembled in the animal potentially inducing both humoral as well as cell-mediated immunity.

In initial efficacy studies an Ad5 vector containing the capsid and $3C^{pro}$ coding regions of the A12 laboratory strain of FMDV protected swine from clinical disease after contact challenge [50]. These studies further demonstrated that an active $3C^{pro}$ was essential for protection. Subsequently an Ad5 vector containing the capsid coding region from a field strain of FMDV, A24 Cruzeiro (Ad5-FMD-A24), was constructed and swine given one dose of the vector were protected from direct inoculation challenge as early as 7 days postvaccination and for up to 42 days [51]. In a similar study cattle given one dose of Ad5-A24 were protected from direct inoculation challenge by 7 days postvaccination [52]. More recently Ad5-FMD vectors containing the capsid from other FMDV serotypes and subtypes have been constructed and successfully tested in cattle (Moraes et al., unpublished data; Grubman et al., manuscript in preparation). These vectors are currently undergoing advanced development as vaccines.

Recently a number of groups have used a variety of systems to express the FMDV capsid in the presence or absence of the $3C^{pro}$ coding regions and in some cases supplemented with various cytokines as potential adjuvants. Recombinant fowlpox or pseudorabies virus vectors or naked DNA administered to swine induced FMDV-specific neutralizing antibody responses and varying degrees of protection [53–56]. Li et al. [54] used a baculovirus-silkworm expression system and demonstrated that 4 of 5 cattle inoculated with the hemolymph from infected silkworms were protected from challenge. Some of these expression systems appear promising, but they are still at earlier stages of testing and development compared to the Ad5-FMD vaccine candidates.

4.3. Live attenuated vaccines

Attempts to develop live attenuated FMD vaccines have met limited success with vaccine viruses showing unstable phenotype or differences in pathogenesis for individual species (e.g. attenuated in cattle but not in swine) or viruses too attenuated to consistently induce protective immune responses [57–60]. These vaccines relied on the use of viruses selected in cell culture or in laboratory animals showing attenuated phenotypes [57]. However, the mechanisms of attenuation were largely unknown, attenuation was incomplete and protective immune responses were not as consistent as with inactivated vaccines. Due to these problems and concerns over reversion to virulence through mutation or recombination with field viruses, live FMD vaccines have not been pursued for many years.

The advent of infectious cDNA technologies and knowledge about FMDV functional genomics allowed the introduction of specific changes in the FMDV genome and evaluation of the attenuating effects of such changes not only in cell culture but also in animals [61,62]. An important milestone in understanding FMD pathogenesis was the discovery that leader protein (L^{pro}) is a viral determinant for virulence. This viral non-structural protein, present in all FMDV serotypes, has been identified as a protease that not only cleaves itself from the nascent viral polyprotein [63], but also cleaves cellular proteins and modulates host innate responses [64–66]. A genetically engineered FMDV serotype A12 lacking the L^{pro}-coding region (A12-LLV2) was attenuated [67] and was not transmissible between cattle or swine, yet it induced an immune response that was partially protective [68,69]. An inactivated antigen vaccine produced using A12-LLV2 induced full protection similar to that induced by the inactivated wild-type A12 [69]. These series of experiments represented the first time that knowledge about a well defined FMDV genetic virulence determinant was utilized to rationally design an attenuated FMD vaccine.

Little is known about other virulence determinants in the FMDV genome. As new virulence determinants are identified new possibilities for attenuated vaccines will arise. Translating this knowledge into vaccine candidates will require detailed knowledge of the virus host interaction and mechanisms of pathogenesis in order to address concerns about complete attenuation in all susceptible species and decreasing the possibility of reversion to virulence.

5. The role for basic research in the rational design of FMD vaccines

Although the currently available inactivated vaccines have a number of positive characteristics, most importantly they induce protection against challenge and thus can prevent and control FMD, there are major shortcomings of this vaccine and its production that remain unaddressed (Table 1). Ad5-FMD vaccines, the first molecular vaccines undergoing advanced development, have successfully addressed some of these short comings but important gaps remain. Some of these limitations are being addressed in the Ad5-FMD platform with number of approaches including the use of

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Table 1
Comparison of product profiles for inactivated and Ad5 FMD vaccines.

Vaccine feature	Inactivated antigen	Ad5 vectored
Effective after one dose	Yes	Yes
Prevents viral shedding	Yes	Yes
Onset of immunity in 7 days	Yes	Yes
Safe production without the use of virulent virus	No	Yes
Stable for long time after formulation	No	Yes
Negative marker—DIVA compatible	Some	Yes
Thermostable	No	No
Cross-serotype protection	No	No
Induces life long protection	No	No
Prevents carrier state	No	No
Confers rapid protection (<7 days)	No	No

traditional adjuvants (e.g. synthetic emulsions) or cytokine-based adjuvants such as type I interferon [70], alternate routes of delivery, tissue targeting, Ad5-FMD vaccines with enhanced capsid stability, and inclusion in the vector of FMDV non-structural protein coding regions that may enhance empty capsid assembly [71].

Additional longstanding shortcomings of FMD vaccines require new approaches based on detailed knowledge of the viral life cycle and the host response to infection and vaccination. Current inactivated FMD vaccine strains have had minimal or no molecular changes other than adaptation for growth in the production cells. This adaptation has proven difficult in some cases with viruses not producing enough yield for vaccine production or the virus changing important antigenic determinants during cell adaptation [72,73]. Infectious cDNA technology could be utilized to improve or change specific regions of the capsid to improve growth, stability and antigenicity. For example Mateo et al. [74] showed that introducing amino acid changes in predicted stabilizing sites in the FMDV capsid resulted in viruses that were more thermostable. Another approach to changing the capsid was taken by Mason and co-workers [75] who engineered an FMDV containing a deletion of the main receptor binding motif in VP1 (RGD). This virus only grows in cells engineered to contain a synthetic viral receptor, but induced a protective immune response in cattle. Despite the promising results these approaches have not found their way into vaccine production systems.

In conclusion vaccines are a fundamental component of strategies aimed at global control and eradication of FMD. It is unlikely that a single vaccine approach will solve the many shortcomings of current vaccines. More likely each situation will require fit-for-purpose vaccine approaches including the currently available inactivated antigens. Also different stages during control and eradication will require the combination of different vaccine strategies. For example enzootic regions will require highly effective vaccines that can induce broadly protective and long-term responses in order to decrease virus transmission and incidence of clinical disease. Eradication might require vaccines that will allow differentiating infected from vaccinated animals (DIVA). Emergency response to outbreaks will require fast acting DIVA compatible vaccines with long-term stability of the formulated ready to use product. These fit-for-purpose, rationally designed vaccine strategies and their companion diagnostic tests will need to be developed based on deep understanding of the functional genomics of FMDV and the mechanisms of viral virulence. Investment in basic research is necessary to obtain the required knowledge and translational research necessary to convert this knowledge into useful products.

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