UNIVERSIDAD DE ANTIOQUIA
1803

Revista Colombiana de Ciencias Pecuarias

New approaches to control foot-and-mouth disease: antivirals and novel vaccines

Marvin J Grubman[1]

[1]United States Department of Agriculture (USDA), Agricultural Research Service (ARS), North Atlantic Area (NAA), Plum Island Animal Disease Center (PIADC), Greenport, NY 11944
marvin.grubman@ars.usda.gov

(Recibido: 1 febrero, 2006; aceptado: 31 mayo, 2006)

Summary

Foot and mouth disease (FMD) is a highly contagious viral-induced disease of cloven-hoofed animals that results in serious economic consequences in affected countries that have a significant international livestock trade. Currently disease control measures include inhibition of susceptible animal movement, slaughter of infected and susceptible in-contact animals, disinfection, and possibly vaccination with an inactivated whole virus antigen. However, there are a number of problems with use of the current vaccine in outbreaks in countries which have been previously free of FMD. As a result countries which vaccinate face a longer delay in regaining FMD-free status than countries which do not vaccinate but rather slaughter all infected or susceptible in-contact animals. Researchers have been attempting to develop both new FMD vaccines to overcome the limitations of the current inactivated vaccine as well as methods to more rapidly induce a protective response. In this manuscript I discuss the most effective new FMD vaccines and novel antiviral strategies that are currently being examined.

Key Words: *antiviral strategies, foot and mouth disease, prevention, vaccines.*

Introduction

Foot and mouth disease (FMD) is a highly contagious disease of domestic and wild cloven-hoofed animals including cattle, swine, sheep, goats, and deer that rapidly replicates in the host and readily spreads to susceptible animals by contact and aerosol (14). The disease is characterized by fever, lameness and vesicular lesions on the tongue, feet, snout and teats resulting in high morbidity but low mortality in adult animals. However, mortality can be high in young animals since the disease can affect the heart (14). FMD is considered one of the most contagious diseases of animal or man and is listed on the A list of infectious diseases of animals by the Office International des Epizooties (OIE), the world organization of animal health. This designation indicates that FMD is a transmissible disease that has the potential for very serious and rapid spread and is of serious socioeconomic importance. A disease outbreak must be immediately reported by a member country to the OIE and results in the inhibition of trade in susceptible animals and their products. Clearly outbreaks of FMD can have devastating economic consequences in countries that have a significant international livestock trade.

FMD is enzootic in all continents except Australia and North America, but by the 1990's had been eradicated from many developed countries (4).

However, numerous outbreaks have occurred in the past decade in countries that had been free of the disease for many years. In particular, outbreaks in Taiwan in 1997 and the United Kingdom in 2001 resulted in the slaughter of millions of animals at a cost of billions of dollars (US) (15, 28). Furthermore, there has been a re-emergence of FMD in some countries in South America that were either free of the disease, such as Uruguay and Argentina, or where specific regions of the country were disease-free such as the southern part of Brazil (9). Currently an outbreak of FMDV type O is occurring in the southern Brazilian states of Mato Grosso do Sul and Parana with concurrent severe economic consequences. In addition, since the terrorist attacks in the U.S. in 2001, the possible use of FMDV as a bioterrorist weapon is of considerable concern to disease-free countries.

The causative agent of the disease, FMD virus (FMDV), the type species of the *Aphthovirus* genus, of the *Picornaviridae* family, contains a single-stranded, positive-sense RNA genome of approximately 8500 bases surrounded by an icosahedral capsid with 60 copies each of 4 structural proteins, VP1-4 (14, 23). The virus is antigenically highly variable and consists of seven serotypes and multiple subtypes (14). Upon infection of cells the virus is uncoated and the viral genome translated into a polyprotein that is processed by the viral encoded proteinases L and 3C and the peptide 2A into the mature viral structural and nonstructural (NS) proteins. The 3C proteinase, $3C^{pro}$, processes the capsid precursor polyprotein P1-2A into the viral structural proteins, VP0, VP3, and VP1 and these capsid proteins assemble, via a series of steps, into the virus particle. Researchers have attempted to utilize this basic information in the development of novel marker vaccines.

Disease control

The methods used to control FMD outbreaks and eliminate the disease have included inhibition of movement of susceptible animals and animal products, slaughter of infected and susceptible in-contact animals, disinfection, and vaccination programs with an inactivated whole virus antigen. As a result of these campaigns many countries including Western European countries and parts of South America became FMD-free in the late 1980's and early 1990's. These countries then stopped vaccination programs and were reluctant to use vaccination in the event of an outbreak because OIE regulations favored slaughter as the most rapid way to regain FMD-free status and thus resume international trade. There were a number of reasons for this policy including difficulty in distinguishing vaccinated from infected animals and the potential for the development of an asymptomatic persistent infection or carrier state upon exposure of vaccinated animals to infectious virus. For these reasons the U.K. did not vaccinate to control the 2001 outbreak, while in the subsequent outbreak in The Netherlands animals were vaccinated but were eventually all slaughtered (21). The large-scale slaughter of infected animals and in-contact susceptible animals, many of which were probably not infected, raised considerable public concern and has resulted in a change in OIE, European Union, and U.K. policy to a more prominent role for vaccination, including vaccination-to-live, in disease control in previously FMD-free countries (2, 25). Included in this policy change was the recognition and support for development of new vaccines and disease control strategies (2).

There are a number of other drawbacks with the use of the current inactivated vaccine in the elimination of FMD from disease-free countries including the requirement for expensive, high-containment facilities for vaccine production and the inability of this vaccine to induce the rapid protection required to control FMD. As a result there is a "window of susceptibility" prior to the onset of vaccine-induced immunity.

Development of new vaccines

Over the past 30 years researchers have attempted to develop alternative vaccines that overcome some of the drawbacks with the inactivated vaccine. To be successful in campaigns to eliminate FMD in a previously disease-free country a new vaccine must induce protection in one inoculation and must contain a marker to allow unequivocal differentiation between vaccinated and infected animals. Thus

far the only vaccine that has met these goals is an FMDV empty viral capsid immunogen delivered by a replication-defective human type 5 adenovirus (Ad5) vector (16, 18).

Empty viral capsids are virus particles lacking nucleic acid and are naturally produced in FMDV infected cell cultures. These particles are antigenically similar to infectious virus, and are as immunogenic as virions in animals (22, 24). Thus, this immunogen contains the entire repertoire of immunogenic sites present on intact virus. Utilizing recombinant DNA technology we have produced an Ad5 construct that contains the portions of the FMDV genome required for capsid protein synthesis and assembly (16). This construct includes the coding region for the viral capsid protein precursor, P1-2A, and $3C^{pro}$ (26), but lacks the coding regions for most of the other viral NS proteins. Furthermore, when assembled, this immunogen, by definition lacks FMDV nucleic acid, and can not synthesize infectious viral nucleic acid when inoculated into animals. This marker vaccine can be utilized with companion diagnostic assays specifically designed to detect FMDV antigens not present in the vaccine and thus vaccinated animals can unequivocally be distinguished from infected animals. An additional advantage of a DNA-based FMD vaccine is the faithful replication of Ad5 and foreign DNA which thereby overcomes the potential problem of selection of antigenic variants during FMDV cell culture passage required for traditional inactivated vaccine production.

We have constructed an Ad5 vector containing the capsid coding region from FMDV A24 Cruzeiro, a field strain from Brazil, and the $3C^{pro}$ coding region (Ad5-A24) (18). Inoculation of swine with one dose of this vaccine protected animals subsequently challenged by direct inoculation 7, 14 or 42 days later from clinical disease, viremia or virus in nasal swabs, while control animals developed severe clinical disease, including fever and viremia (18). In a separate experiment swine were inoculated with Ad5-A24 and challenged by direct inoculation 5 days later (17). Although all vaccinated animals developed clinical disease, their lesion score was considerably less than control challenged animals and 2 of the 3 vaccinated animals did not develop viremia, while the third animal in this group only had a low level viremia for 1 day. In contrast, all the control animals developed viremia for 2-3 days. Thus, Ad5-A24 can induce a partially protective response in swine within 5 days.

Cattle inoculated with a single dose of the Ad5-A24 vaccine were protected from systemic disease and viremia when challenged 7 days later by intradermolingual inoculation and contact exposure (19). In addition, preliminary experiments indicate that this vaccine can be administered more than one time since twice-inoculated animals developed a significant boost in the FMDV-specific neutralizing antibody response. Thus, Ad5 vectored FMDV may be useful in vaccination campaigns in countries where FMD is enzootic. Furthermore, serological evidence from the above experiment indicates that after two inoculations with this vaccine there is still a clear distinction of vaccinated animals from infected animals (13).

FMDV serotype O is prevalent worldwide and has caused recent outbreaks in Asia, Europe and the current outbreak in Brazil. It has been shown that the inactivated O vaccine is a poorer immunogen than a serotype A vaccine and successful O vaccines require 4-5 times greater antigenic payload than A (10, 20). We constructed a serotype O1 Campos Ad5 vaccine and inoculated pigs once with the same dose as Ad5-A24 and challenged the animals 21 days later (5). The Ad5-O1C vaccine induced a detectable, but low, FMD-specific neutralizing antibody response. Vaccinated animals were not completely protected, but disease was delayed and considerably reduced and there was a 10.000-fold reduction in viremia as compared to control animals. We are currently examining various avenues to enhance the efficacy of the Ad5-O1C vaccine.

Development of antivirals for rapid protection

While both the traditional vaccine and the Ad5-A24 vaccine can induce protection by 7 days postadministration (11, 19), the ability of FMDV to replicate and spread very rapidly results in a "window

of susceptibility" to disease prior to the induction of the adaptive immune response.

Antivirals can potentially cover this early period of susceptibility since these compounds can rapidly inhibit virus replication. Antivirals can either be designed to specifically inhibit the function of one or more viral proteins or a more generic antiviral approach can be attempted utilizing components of the host innate immune response.

One of the initial general responses of the host to viral infection is the induction of type I interferon (IFN−α/β) mRNA (3). Expression, secretion, and binding of IFN−α/β protein to specific receptors on cells results in initiation of a signal transduction pathway and induction of a virus-resistant state in these cells by activation of a series of genes whose protein products can inhibit various steps in the virus life cycle (3, 12). Although viruses, including FMDV, have devised various strategies to overcome the host antiviral response, we and others have shown that FMDV replication is inhibited in cell cultures pretreated with IFN-α/β protein (1, 6, 8, 12). These results suggest the potential use of IFN-α/β as an anti-FMDV agent in animals.

We have used the replication-defective Ad5 vector system as an efficient method of introduction and expression of IFN-α/β in swine (7, 17). In this delivery system IFN is produced in the animal and the amount of IFN expressed can be controlled by the dose of Ad5 administered. This approach overcomes some of the current concerns with the clinical use of IFN including its rapid metabolism in vivo requiring frequent high doses. Recombinant Ad5 vectors containing either porcine IFN-α or porcine IFN-β were constructed and expressed high levels of biologically active IFN in cell culture (7). Swine were inoculated with Ad5-IFNα or a control Ad5 virus and challenged by direct inoculation one day later with FMDV (7). The group given Ad5-IFNα did not develop clinical disease or viremia, had only a low FMDV-specific neutralizing antibody response and no antibodies against the viral NS proteins while the control Ad5 inoculated group developed severe clinical disease. In additional experiments the single dose of Ad5-IFNα could protect swine from FMDV for as long as 3-5 days (17). These results indicate that prophylactic treatment with IFN can induce sterile protection. In an additional experiment Ad5-IFNα was administered therapeutically one day postchallenge. The animals had reduced levels of viremia and severity of disease as compared to controls suggesting that this treatment can reduce the amount of virus shed thereby potentially limiting disease spread. In support of this result we showed that treatment of FMDV-infected cells with IFN-α at various times postinfection significantly reduced virus yield (8).

To address the need to induce both rapid and relatively long-lasting protection, we inoculated swine with a combination of Ad5-pIFNα and Ad5-A24 and challenged them 5 days later (17). These animals had a low level, but detectable neutralizing antibody response prior to challenge, and a significant boost in titer after challenge. However, there was no evidence of virus replication and the group was completely protected from clinical disease. These results indicate that the combination approach does afford animals exposed to FMDV soon after treatment both rapid protection and a more robust neutralizing antibody and protective response than animals administered either the antiviral or vaccine alone.

Prophylactic use of IFN was partially effective in cattle, but most of the inoculated animals developed clinical disease which was delayed and less severe as compared to the controls (27). Evidence suggests that the reduced effectiveness of IFN treatment in cattle is a result of a lower blood concentration of IFN than in swine.

How do we propose to implement a combination vaccine and antiviral strategy?

Animals in an FMD-infected zone should be rapidly given an antiviral, but these animals would still be slaughtered. This strategy should reduce the amount and duration of virus shedding. Animals in the surrounding zone free of disease would be gi-

ven both the antiviral and the vaccine and if these animals were negative by a companion diagnostic assay, ie., 3D ELISA, they would not be slaughtered. This strategy should promote a policy of vaccination-to-live thereby reducing the number of animals that would have to be destroyed as well as have a positive environmental and social impact as compared to a slaughter only approach. It is also possible that this strategy can be used in the final phases of FMD eradication campaigns in countries where the disease is enzootic. In particular, the use of a marker vaccine should allow unequivocal demonstration of absence or presence of infected animals during serological surveillance programs.

Resumen

Nuevas aproximaciones hacia el control de la fiebre aftosa: antivirales y nuevas vacunas

La fiebre aftosa es una enfermedad viral altamente contagiosa, que afecta a los animales de pezuña hendida y produce pérdidas económicas importantes, afectando el comercio internacional pecuario de los países que la padecen. En la actualidad las medidas de control para esta enfermedad incluyen la restricción del movimiento de animales susceptibles, el sacrificio de animales infectados y aquellos susceptibles que entran en contacto con los infectados, la desinfección y la posible vacunación con un antígeno viral completo inactivado. No obstante, existen varios problemas con el uso de las vacunas disponibles durante los brotes en países que habían sido considerados previamente libres de la enfermedad. Como resultado de lo anterior, los países que vacunan, enfrentan una demora significativa en obtener nuevamente el estado de "país libre" de fiebre aftosa, a diferencia de aquellos que no vacunan y deciden sacrificar todos sus animales infectados o los susceptibles que se exponen a la infección. Los científicos han estado tratando de desarrollar nuevas vacunas para superar las limitaciones de las actuales vacunas inactivadas, a la vez que se intentan desarrollar nuevos métodos para inducir una respuesta inmune protectora más rápida. En este documento se discuten, nuevas vacunas que son más efectivas para controlar la fiebre aftosa e igualmente, estrategias antivirales novedosas que están actualmente en investigación.

Palabras calve: *estrategias antivirales, fiebre aftosa, prevención, vacunas.*

References

1. Ahl R, Rump A. Assay of bovine interferons in cultures of the porcine cell line IB-RS-2. Infect Immun 1976; 14:603-6.

2. Anonymous. Infectious disease in livestock. 2002. The Royal Society.

3. Biron CA, Sen GC. Interferons and other cytokines. In: Field's Virology. Knipe, DM, Howley, PM (eds). Lippincott Williams & Wilkins, PA, USA 2001; 321-51.

4. Brown F. New approaches to vaccination against foot-and-mouth disease. Vaccine 1992;10:1022-6.

5. Caron L, Brum MCS, Moraes MP, Golde WT, Arns CW et al. Granulocyte-macrophage colony-stimulating factor does not increase the potency or efficacy of a foot-and-mouth disease virus subunit vaccine. Pesq Vet Bras 2005;25(3):150-8.

6. Chinsangaram J, Koster M, Grubman MJ. Inhibition of L-deleted foot-and-mouth disease virus replication by alpha/beta interferon involves double-stranded RNA-dependent protein kinase. J Virol 2001;12:5498-5503.

7. Chinsangaram J, Moraes MP, Koster M, Grubman MJ. A novel viral disease control strategy: adenovirus expressing interferon alpha rapidly protects swine from foot-and-mouth disease. J Virol 2003;77:1621-5.

8. Chinsangaram J, Piccone ME, Grubman MJ. Ability of foot-and-mouth disease virus to form plaques in cell culture is associated with suppression of alpha/beta interferon. J Virol 1999;73:9891-8.

9. Correa Melo E, Saraiva V, Astudillo V. Review of the status of foot and mouth disease in countries of South America and approaches to control and eradication. Rev sci tech Off int Epiz. 2002;21:429-36.

10. Doel TR, Williams L, Barnett PV. Emergency vaccination against foot-and-mouth disease: rate of development of immunity and its implications for the carrier state. Vaccine 1994;12:592-600.

11. Golde WF, Pacheco JM, Duque H, Doel T, Penfold B, et al. Vaccination against foot-and-mouth disease virus confers complete clinical protection in 7 days and partial protection in 4 days: Use in emergency outbreak response. Vaccine 2005;23:5775-820.

12. Goodbourn S, Didcock L, Randall RE. Interferons: cell signalling, immune modulation, antiviral response and virus countermeasures. J Gen Virol 2000;81:2341-64.

13. Grubman MJ. Development of novel strategies to control foot-and-mouth disease: Marker vaccines and antivirals. Biologicals 2005;33:227-34.

14. Grubman MJ, Baxt B. Foot-and-mouth disease. Clinical Micro Rev 2004; 17:465-93.

15. Knowles NJ, Samuel AR, Davies PR, Kitching RP, Donaldson AI. Outbreak of foot-and-mouth disease virus serotype O in the UK caused by a pandemic strain. Vet Rec 2001;148:258-9.

16. Mayr GA, Chinsangaram J, Grubman MJ. Development of replication-defective adenovirus serotype 5 containing the capsid and 3 C protease coding regions of foot-and-mouth disease virus as a vaccine candidate. Virology 1999;263:496-506.

17. Moraes MP, Chinsangaram J, Brum MCS, Grubman MJ. Immediate protection of swine from foot-and-mouth disease: a combination of adenoviruses expressing interferonalphaandafoot-and-mouthdiseasevirussubunit vaccine. Vaccine 2003;22:268-79.

18. Moraes MP, Mayr GA, Mason PW, Grubman MJ. Early protection against homologous challenge after a single dose of replication-defective human adenovirus type 5 expressing capsid proteins of foot-and-mouth disease virus (FMDV) strain A24. Vaccine 2002;20:1631-9.

19. Pacheco JM, Brum MCS, Moraes MP, Golde WT, Grubman MJ. Rapid protection of cattle from direct challenge with foot-and-mouth disease virus (FMDV) by a single inoculation with an adenovirus vectored FMDV subunit vaccine. Virology 2005;337:205-9.

20. Pay TW, Hingley PJ. Correlation of 140S antigen dose with the serum neutralizing antibody response and the level of protection induced in cattle by foot-and-mouth disease vaccines. Vaccine 1987;5:60-4.

21. Pluimers FH, Akkerman AM, van der Wal P, Dekker A, Bianchi A. Lessons from the foot and mouth disease outbreak in the Netherlands in 2001 Rev sci tech Off int Epiz 2002;21:711-721.

22. Rowlands DJ, Sangar DV, Brown F. A comparative chemical and serological study of the full and empty particles of foot-and-mouth disease virus. J Gen Virol 1974;26:227-38.

23. Rueckert RR. Picornaviridae: the viruses and their replication., p. 609-654. *In* B. N. Fields, D.M. Knipe, and P.H. Howley (ed.), Field's Virology. Lippincott-Raven, Philadelphia and New York, 1996.

24. Rweyemamu MM, Terry G, Pay TW. Stability and immunogenicity of empty particles of foot-and-mouth disease virus. Arch Virol 1979;59:69-79.

25. Scudamore JM, Harris DM. Control of foot and mouth disease: lessons from the experience of the outbreak in Great Britain in 2001. Rev sci tech Off int Epiz 2002;21:699-710.

26. Vakharia VN, Devaney MA, Moore DM, Dunn JJ, Grubman MJ. Proteolytic processing of foot-and-mouth disease virus polyproteins expressed in a cell-free system from clone-derived transcripts. J Virol 1987;61:3199-207.

27. Wu Q, Brum MCS, Caron L, Koster M, Grubman MJ. Adenovirus-mediated type I interferon expression delays and reduces disease signs in cattle challenged with foot-and-mouth disease virus. J Int Cyt Res 2003;23:371-80.

28. Yang PC, Chu RM, Chung WB, Sung HT. Epidemiological characteristics and financial costs of the 1997 foot-and-mouth disease epidemic in Taiwan. Vet Rec 1999;145:731-4.