PAST AND PRESENT VACCINE DEVELOPMENT STRATEGIES FOR THE CONTROL OF FOOT-AND-MOUTH DISEASE

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Summary. – Foot-and-mouth disease (FMD) virus (FMDV) was the first animal virus to be identified. Since then, it has become a model system in animal virology and more information has been obtained about FMDV. The disease causes heavy economic crises in enzootic countries both due to loss of animal health and productivity. The only way of its control in an enzootic area is strict vaccination and restricted animal movement. The first experimental vaccine against FMD was made in 1925 using formaldehyde inactivation of cattle tongue infected with the virus and this approach remained the basic one until late 1940s. Antigenic plurality and continuous co-circulation of different serotypes in a given geographical region and persistence of virus in infected or vaccinated animals make the disease very difficult to control. The latter is solely based upon the application of isolation, slaughter or aphtisation, and vaccination. With the advent of recombinant DNA technology, recombinant protein and/or DNA-based vaccines are being tested in various heterologous systems for development of FMD vaccines. The subunit vaccines, synthetic peptide vaccines, DNA vaccines, cytokine-enhanced DNA vaccines, recombinant empty capsid vaccines, chimeric viral vaccines, genetically engineered attenuated vaccines, recombinant viral vector vaccines, self-replicating genetic vaccines and transgenic plants with expressed FMDV proteins represent the present vaccine development strategies for control of FMD.

Key words: foot-and-mouth disease; Foot-and-mouth disease virus; vaccine; development; strategies

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1. Introduction

FMD is the most feared, highly contagious vesicular disease of cloven-hoofed animals (Brown, 2003). It can have a severe impact on the productivity of domestic livestock, and because of its ability to spread very rapidly in susceptible populations, the countries free of the disease attempt to protect their status by maintaining strict control of import of live animals and animal products (Barnett *et al.,* 2002).

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Abbreviations: APC = antigen-presenting cells; BEI = binary ethylene imine; BHK-21 = Baby hamster kidney-21; CMV = Cytomegalovirus; FMD = foot-and-mouth disease; FMDV = FMD virus; GM-CSF = granulocyte-macrophage-colony stimulating factor; IFN = interferon; IL = interleukin; PKR = protein kinase; RGD = arginine-glutamate-aspartate; SAT = South African Territory; wt = wild type

FMD is a major constraint to international trade and its presence can dramatically reduce the export potential of the agricultural sector. The consequences of its introduction into a previously FMD-free country in which agricultural exports contribute significantly to total foreign earnings are particularly damaging (Barnett *et al.,* 2002) as illustrated by the outbreaks in 2001 in UK (Scudamore and Harris, 2002).

The causative agent, FMDV (the species *Foot-and-mouth disease virus*, the genus *Aphthovirus,* the family *Picornaviridae,* Racaniello (2001)) exists in seven antigenically distinct serotypes, namely O, A, C, Asia1, and SAT 1, 2, and 3 (Domingo *et al.,* 2003; Knowles and Samuel, 2003), of which types A, O, C and Asia1 are circulating in India. FMDV genome consists of a single-stranded positive-sense RNA of 8,500 b that is enclosed in an icosahedral capsid. The latter contains four structural proteins, namely VP4, VP2, VP3, and VP1 in that order (Acharya *et al.,* 1989), which are secondary cleavage products of P1 polyprotein, a primary cleavage product of a 250 K polyprotein encoded by the genome (Sanger *et al.,* 1977).

The countries involved in the control of FMD usually regard virus eradication from the territory as their final goal. The countries of entire Western Europe and parts of South America have followed such a strategy and eradicated the disease from their territory. Even a century after the Loeffler and Frosch (1898) discovery of FMD the latter is still persisting as a global menace. Worldwide eradication should be the ultimate goal, because the existence of the disease anywhere on the globe keeps the rest of the countries at risk. Slaughtering the affected animals, ring, frontier or barrier vaccination can prevent the spread of infection (Brown, 1999). Many international regulations impose embargo on import of animals; their meat and milk products from endemic countries to minimize the risk of disease introduction into FMD-free countries (Brown, 1999).

A buffer zone containing vaccinated animals may be used to separate the area within an endemic country from the FMD-free area, from which cattle and its products are exported (Kitching, 2002a). In countries that are endemic for FMD, sheep may be included in regular vaccination campaigns, although they are rarely vaccinated more than once a year (Kitching and Hughes, 2002). The immune response to FMD vaccine in young pigs is poor. Protection against this disease is best provided by vaccinating pregnant sows so that the immunity will be passed onto piglets through colostrums (Kitching and Salt, 1995).

Strict control measures like stamping out the affected animals, restriction on movement of animals and animal products from endemic areas to the disease-free zones have led to the eradication of the disease from the North America, parts of Europe and Australia. Antigenic plurality of FMDV arising due to the quasi-species nature (Domingo *et al.,* 1985), its extreme contagiosity, wide geographical distribution, prevalence of a large number of strains within a serotype, and occurrence of virus persistence and carrier status associated with short duration of the immunity makes the FMD control difficult (Domingo *et al.,* 1980).

Countries endemic for FMD attempt to eradicate the disease with programs that utilize vaccines prepared from chemically inactivated FMDV (Barteling and Vreeswijk, 1991). The countries free from the disease or in the last stages of eradication run constant risk of the disease (Chen *et al.,* 1999). Effective control of the disease in an endemic country like India needs better vaccines along with strong diagnostic support. The disease occurs in all parts of the country throughout the year. Majority of outbreaks are due to type O, followed by A_{22}, and Asia1 (Tosh *et al.,* 2003). FMD control and eradication is one of the priorities of animal health programmes in India. Slaughter, which is not feasible owing to the socio-economic conditions, leaves vaccination as the only means for the effective control of FMD outbreaks (Balamurugan, 2002). Since presence of multiple serotypes and lack of cross-protection among the involved serotypes necessitates the use of polyvalent vaccines to confer a complete protection. Hence the only approach to control the disease in India is implementation of a regular vaccination programme with periodic updating of vaccine virus strains.

Although inactivated virus vaccines are effective as part of eradication programmes in endemic areas, vaccine production and application may cause a number of problems limiting the vaccination realized within the emergency control program. Namely, it may leave a residual live virus in vaccines (Beck and Strohmaier, 1987), induce antibodies to some viral non-structural proteins, interfere with the detection of infected or vaccinated animals that harbour the virus and display subclinical disease, result in presence of many antigenically distinct virus subtypes and emergence of new variants that could be used for vaccine production only after adaptation to cell cultures, and lead to poor quality vaccines inducing a short duration immunity (Grubman and Mason, 2002). The current vaccines do not prevent infection and animals from becoming long-term virus carriers (Salt, 1993). Current evidence suggests that low level of infection with FMDV does not induce detectable levels of antibodies to non-structural proteins, which makes detection of carrier animals difficult (Kitching, 1998).There are several ways of potential improvement of conventional vaccines, for example development of vaccines of high thermostability; broader immunogenicity, life-long immunity; resistant to maternal antibodies; not resulting in carrier state; enabling differentiation of vaccinated and naturally infected animals, and applicable by topical routes (Barnett *et al.,* 2002).

Currently, many of the countries are gradually moving towards holding strategies of FMD vaccines in the event of an outbreak. All the countries rely primarily on slaughter, restrictions on animal movement and zoological-sanitary

measures (Sobrino *et al.,* 2001; Barnett and Carabin, 2002). The reserves consist of stored vaccines that are replaced every 12 to 18 years or concentrated antigens stored indefinitely over liquid nitrogen, which can be rapidly formulated into vaccines in the event of need (Sobrino *et al.,* 2001). Some of good examples of such reserves are the North American Vaccine Bank, the International Vaccine Bank and the European Union Vaccine Bank etc. The largest bank is probably the latter, which holds up to 5 million cattle dose equivalents of most of the major vaccine strains including the SAT serotype (Garland, 1997). Emergency vaccines are often of high potency to ensure both rapid protective immunity and great cross-specificity. Additionally, such vaccines also appear to be able to reduce local virus replication in the oropharynx thereby limiting transmission of the disease to other susceptible animals (Salt *et al.,* 1998; Cox *et al.,* 1999). Development of innate immune response following emergency vaccination against FMD in pigs has been reported (Rigden *et al.*, 2003). Barnett *et al.* (2004) showed for the first time that a high potency FMD vaccine is capable of inhibiting local virus replication and consequently persistence and a carrier state in sheep.

2. Challenges in FMD eradication

2.1 Antigenic variation in FMDV

RNA viruses in general, and FMDV in particular, exhibit very high mutation rates, in the range of 10^{-3} to 10^{-5} per nucleotide per genome replication (Domingo and Holland, 1988; Drake and Holland, 1999). The presence of seven serotypes and multiple subtypes (Grubman and Baxt, 2004), antigenic complexity and frequent emergence of antigenic variants that often co-circulate in a given geographical area (Mateu *et al.,* 1988), which has led to extreme genetic heterogeneity or the quasi-species concept (Domingo *et al.,* 1985) are the problems encountered in the design of an effective vaccine (Mateu *et al.,* 1994) and control of the disease by vaccination (Kitching *et al*., 1988). Antigenic variation occurs even in cell or tissue culture systems either in the presence or absence of antibodies (Borrego *et al.,* 1993; Domingo *et al.,* 1993; Manoj Kumar *et al.,* 2003). This has implications for vaccine production, since a number of tissue culture passages are required to produce vaccine for a new variant, leading to the possibility the vaccine virus eventually does not provide complete antigenic coverage (Grubman and Baxt, 2004).

2.2 Persistence of FMDV

FMDV persists in the cells of basal layer of pharyngeal epithelium, particularly of the dorsal soft palate (Zhang and Kitching, 2001). Ruminant animals that had recovered from infection with FMDV and vaccinated ruminants that had contact with live virus may retain infection in the pharyngeal region for variable period of time (Kitching, 2002c). The asymptomatic infection in cattle, buffaloes, sheep, and goats may be the outcome of an acute infection established by pharyngeal or nasal exposure to the virus or a result of immunization with live attenuated vaccine (Sutmoller and Gaggero, 1965). The plurality of FMDV (Domingo *et al.,* 1985) leads to virus persistence in the animals by an unknown mechanism. Analysis of persistently infected recovered cattle revealed heterogeneity of viral populations (Gebauer *et al.,* 1988). The viral persistence and carrier status in convalescent and in recovered animals make them main reservoirs, though it is extremely difficult to demonstrate the disease transmission from one to another animal under controlled conditions, and only circumstantial evidence suggests involvement of persistently infected animals in the spread of the disease (Salt, 1993). The duration of the carrier state depends on the species and individual. The African Cape buffalo may carry virus for more than five years, cattle for over three years, sheep for up to nine months (Salt *et al.,* 1996), goats and wild ruminants for shorter periods of time, while for South American camelids no carrier state exists (David *et al.,* 1993). The crucial problem is that there are no currently available diagnostic tests sensitive enough to detect persistently infected animals with full certainty (Kitching, 2002b).

3. New approaches in FMD vaccine development

As an alternative attempt to avoid the use of live virus, while retaining the myriad of viral antigenic determinants, several works had been carried out for the development of new generation vaccines. With the advent of molecular approaches to FMD vaccination especially the recombinant DNA technology, recombinant protein-based vaccines for FMD are being developed in bacteria, baculoviruses and transgenic plants (Brown, 1999). However, these vaccines have so far progressed to the point at which they might offer sufficient advantages over existing conventional vaccines regards production methodology, bio-security, stability, antigenic spectrum, speed and duration of the immune response, cost that would justify the time and brain work invested, large scale production, testing and registration (Brown, 1999; Sobrino *et al.,* 2001; Barnett *et al.,* 2002). They have shown only partial protection with shorter duration of immunity due probably to the lack of T cell epitopes in the expressed protein, improper folding of the peptide or combination of both (Sobrino *et al.,* 2001). Emergence of nucleic acid-based vaccines has paved the way for the development of so-called 3rd generation vaccines. The limitations of conventional vaccines and the different approaches that are being used to develop alternative safe and effective vaccine strategies are summarized in Table 1.

Table 1. Potential advantages and disadvantages of FMD vaccines

Vaccine strategy	Thermostability	Safety in productiom	Immunogenicity	Cost	Duration	Potency	Differentiation between infected and vaccinated animals
Conventional inactivated cell culture vaccines	Low	Inadequate	High	High	Limited	Partial	Limited
Viral subunits and synthetic peptides vaccines	High	High/excellent	Low	Variable	*	*	Good
Recombinant empty capsid vaccines	Low	High	High	Variable	*	*	Good
DNA vaccines and recombinant viral vectors vaccines	High for DNA vaccines, low for viral vector vaccines	Good	Potentially high	Low	*	*	Good
Genetically engineered attenuated vaccines	Low	Variable and unpredictable	High	Low	*	*	Potentially good

*Detailed data not available and requiring further study.

As discussed below, although much has been learned from FMDV research, it is clear that better understanding of mechanisms of protection against FMD and immune evasion by the virus is a prerequisite of designing new more effective vaccines. Principles of some new approaches to the preparation of FMD vaccines are shown in Fig. 1.

3.1 Subunit vaccines

Early observations indicate that isolated VP1 and the fragments derived from its C-terminal half are the only viral capsid products capable of inducing neutralizing antibodies and conferring partial protection (Bachrach *et al.,* 1975; Strohmaier *et al.,* 1982; Meloen *et al.,* 1986). Immunization with VP1 produced in bacteria conferred protection in the pig (Kleid *et al.,* 1981). However, the immunogenicity of such a VP1 was lower by several orders of magnitude than that of the equivalent amount of antigen incorporated in viral particles (Brown, 1988, 1992; Domingo *et al.,* 1990). to use as a vaccine has been carried out. The vaccine based on cloned and expressed VP1 of serotype Asia1 (Suryanarayana *et al.,* 1985) gave a partial protection (Suryanarayana *et al.,* 1992). Because of high of experiments in natural hosts, most of the work with experimental vaccines was carried out in mice and guinea pigs. These experiments provided interesting and significant insights into the response of the host to FMDV antigens (Suryanarayana *et al.,* 1992). Until now recombinant vaccines produced in prokaryotic expression systems were only partially successful (Suryanarayana *et al.,* 1992; Bayry *et al.,* 1999). This could be due to non-native form (e.g. improper folding) of proteins expressed inside the cell and the need for other viral structural proteins to elicit optimum neutralizing antibody response. Bayry *et al.* (1999) have studied a immuno-reactive recombinant peptide expressed in *E. coli* encoding the C-terminal portion of VP1 of FMDV type Asia1 with various

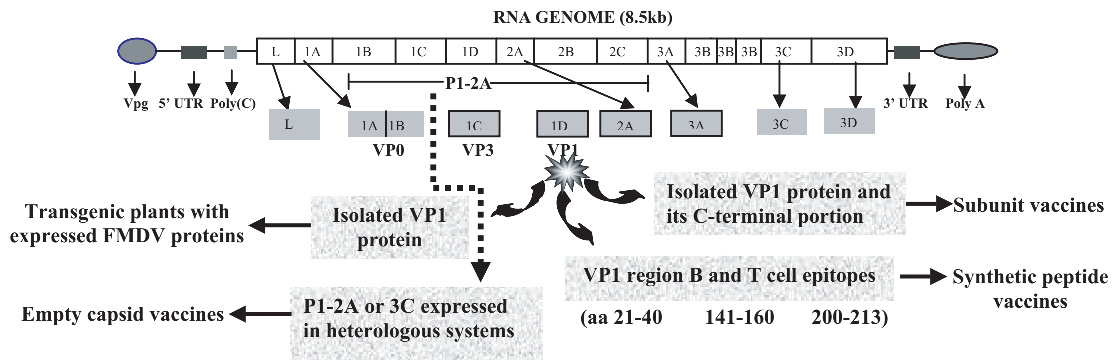

Fig. 1

FMDV genome and strategies of development of FMD vaccines

adjuvants in guinea pigs and showed that the neutralizing antibody titer varied with the nature of the adjuvant. On the other hand, swine vaccinated with recombinant *E. coli*-derived VP1 elicited effective immune response and became protected from viral challenge (Wang *et al.,* 2003).

3.2 Synthetic peptide vaccines

Numerous studies carried out with respect to synthetic peptides representing immunogenic regions deduced by various methods including monoclonal antibodies (MAbs) have led to elucidation of T and B cell epitopes which in turn have provided a better understanding of humoral and cellular immune responses to FMDV (Brown, 1988; Domingo *et al.,* 1990; Collen, 1994; Mateu, 1995). The results predicted that the VP1 region of aa 21–40 contains T-cell epitopes and offers cross-protection to serotypes, the VP1 region of aa 141–156 protects homologous virus strains, and the region of aa 200–213 offers cross-protection to homologous and heterologous viruses (Strohmaier *et al.,* 1982). Synthetic peptides corresponding to C-terminal region of VP1 (aa 147–160 and 200–213) that could elicit high neutralizing antibody response were tested as vaccines in guinea pigs (Strohmaier *et al.,* 1982). In the following years, the concept that immunogenic peptides should also include viral T cell epitopes to provide adequate co-operation with immune B lymphocytes to induce an effective neutralizing antibody response, became generally accepted (Francis *et al.,* 1987; Domingo *et al.,* 1990; Collen *et al.,* 1991; Brown, 1992; Rowlands, 1994; Mateu, 1995; Meloen *et al.,* 1995; Sobrino *et al.,* 1999). A synthetic peptide designed against the G-H loop of VP1 protein, when tested in a large scale vaccination study in cattle (Taboga *et al.,* 1997), was immunogenic, but the vaccinated animals were poorly protected from the disease and antigenic variants of the challenge virus appeared in many of the unprotected animals. A peptide construct consisting of a virus-specific T-helper epitope within the aa 170–188 sequence of VP1 and the main antigenic region of FMDV A_{22} (aa 135–158) elicited a high protective antigenic, immunogenic and T cell proliferative activity (Volpina *et al.,* 1999). A tandem peptide containing the T cell peptide 3A (aa 21–38 aa) and B cell antigenic site VP1 (aa 137–156) efficiently stimulated lymphocytes from infected animals *in vitro* (Blanco *et al.,* 2001). Wang *et al.* (2001) have reported effective control of FMD using a synthetic peptide-based vaccine. Chemically synthesized tandem repeats of immuno-dominant region of VP1 and the T cell epitope of VP4 of FMDV type Asia1 were used as immunogen in guinea pigs (Zhang *et al.,* 2002a). Wang *et al.* (2002) have suggested a safe chemically-defined peptide containing the G-H loop domain of VP1 and a novel promiscuous T-helper (Th) site for broad immunogenicity in multiple species as an advantageous vaccine against FMD.

3.3 DNA vaccines

In recent years, DNA vaccination (also known as genetic immunization, gene vaccination or nucleic acid vaccination) has emerged as one of the most promising applications of recombinant DNA technology. The first two reports indicating potential value of DNA vaccines have appeared in 1993 (Robinson *et al.,* 1993; Ulmer *et al.,* 1993). Immunization with naked DNA can elicit humoral and

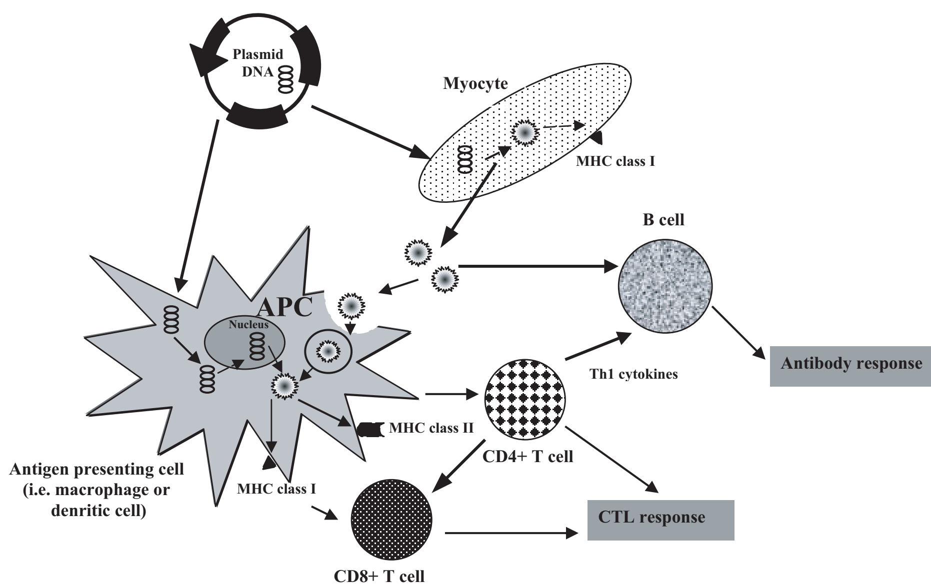

Fig. 2

Mechanisms of DNA-induced immune response to FMDV proteins

cellular immune responses and protection against different pathogens (Wolff *et al.,* 1990). The concept is based simply on inoculation of plasmid DNA, which encodes microbial gene or genes (Sasaki *et al.,* 1999) under a strong eukaryotic promoter like the early CMV promoter and enhancer (Atkins et *al.,* 1999). The mechanisms of immune response produced by DNA vaccine are shown in Fig. 2.

The characteristic advantages of DNA vaccines over live attenuated and the whole killed vaccines have been reported in detail earlier (Sasaki *et al.,* 1999; Dunham, 2002). Initial experiments by Ward *et al.* (1997) revealed that DNA vaccine candidate against FMD was not as effective as the inactivated whole virus vaccines. The elicited neutralizing antibody response fell short of that required for protection against the disease in swine. Chinsangaram *et al.* (1998), have evaluated a candidate DNA vaccine against FMD designed to produce viral capsids lacking viral nucleic acid. Plasmid DNAs containing a portion of the FMDV genome coding for the precursor capsid protein (P1-2A) and wild type (wt) or mutant viral protease 3C induced FMDV antibodies in mice in both the instances. However, only the plasmid DNA containing wt3C elicited a neutralizing antibody response and protected only partially against virus challenge. A DNA vaccine containing an attenuated full-length infectious FMDV clone (deleted L gene and RGD receptor-binding site) induced protection in swines (Ward *et al.,* 1997). Subsequently, demonstrated that their A construct consisting of P1 and 3D sequences and wt3C protected swines against FMD, while a similar construct with mutated 3C lacking post-translation processing ability failed to give protection (Beard *et al.,* 1999). The use of a recombinant protein and DNA vaccine against FMDV type Asia1 infection in guinea pigs has been reported by Zhang *et al.* (2003a). An immune response has been observed in mice inoculated with plasmid DNAs containing multiple epitopes of FMDV (Zhang *et al.,* 2003b). A plasmid (pCEIS) encoding only two major epitopes of FMDV (aa 141–160 and 200–213) protected swines against FMDV challenge (Wong *et al.,* 2000). A plasmid encoding all the structural proteins and processing enzymes gave partial protection against FMDV challenge in pigs (Benvenisti *et al.,* 2001). Shieh *et al.* (2001) have reported that treating animals with DNA plasmids for priming and with FMDV antigen(s) for boosting may elicit immunity to FMDV and that CpGODN motifs may augment this immune response.

3.4 Cytokine-enhanced DNA vaccines

DNA vaccine-induced immune responses can be enhanced or modulated by co-administration of stimulatory molecules as pro-inflammatory cytokines, tumor necrosis factor (TNF), granulocyte-macrophage colony stimulating factor (GM-CSF) and Th1 and Th2 cytokines (Kim *et al.,* 2001). As biological activity of cytokine is difficult to maintain under normal conditions, their application is limited. However, recombinant DNA technology facilitates the use of cytokines in vaccination by manipulating immune responses. Inclusion of cytokines and studying their immuno-modulatory effect is a step forward in the development of new generation vaccines. Stimulation of local pharyngeal immune response could be today achieved by using one of the ever increasing number of identified cytokines, which could succeed in eliminating the virus (Kitching, 2002b).

Cedillo-Barron *et al.* (2001) have reported an enhancement of immune response by co-inoculation of a GM-CSF construct and a FMDV P1-2A3CD construct. Wong *et al.* (2002) have reported a similar enhancement by employing swine interleukin-2 (IL-2) as a gene adjuvant. Although protection against FMDV correlates in general with neutralizing antibodies, a T cell response is essential for effective immunity and ultimate elimination of carrier status in reconvalescing animals. IFN-γ has been reported as a potent antiviral cytokine (Costa-Pereira *et al.,* 2002) stimulating strong T cell responses. Efficacy of cytokines as gene adjuvants has been ascertained by several authors (Cedillo-Barron *et al.,* 2001; Costa-Pereira *et al*., 2002; Wong *et al*., 2002; Kitching, 2002b). The induction of monocytic cell activity, demonstrable by the production of IL-6, IL-8 and IL-12, appears to play a critical role in FMDV emergency vaccine induction of innate immune defense, which relates to early protection against FMD (Barnett *et al.,* 2002). Zhang *et al.* (2002b) have described various stimulatory effects of IFN-γ on replication of FMDV especially in persistently infected bovine cells. A pig inoculated with replication-defective human Adenovirus type 5 vectors containing IFN and FMDV genes was completely protected when challenged 24 hrs later with FMDV. These animals neither exhibited clinical signs of FMD nor developed antibodies against viral non-structural proteins, suggesting that complete protection from infection was achieved (Chinsangaram *et al.,* 2003). Cedillo-Barron *et al.* (2003) have investigated the immune response in pigs to two recombinant plasmids co-expressing immuno-dominant neutralizing antibody epitopes of FMDV VP1 protein (serving as a source of T cell epitopes in induction of immunity) and viral non-structural proteins. Additional co-immunization with plasmids encoding GM-CSF did not result in any change in the immune responses.

3.5 Recombinant empty capsid vaccines

Experimental FMD vaccines have targeted immunogens containing entire repertoire of immunogenic sites present on intact virus but lacking infectious nucleic acid (Grubman *et al.,* 1985,1993; Roosien *et al.,* 1990; Lewis *et al.,* 1991). It has been shown that empty capsids of FMDV retained

antigenicity and immunogenicity (Rowlands *et al.,* 1975) of infectious virus particles and the immunogenicity was superior to that of inactivated virus as the antibodies were produced not only against sequential but also conformational epitopes. Chemically inactivated viral particles and empty capsids have been shown to elicit neutralizing antibody response (Rweyemamu *et al.,* 1979). Production of empty capsids from whole virus is cumbersome. However, it can be achieved by cloning and expression of capsid proteins (P1 coding sequences). Empty capsids were obtained by processing of P1 either *in vitro* in a mammalian, insect and bacterial cells (Grubman *et al.,* 1985; Roosien *et al.,* 1990; Belsham *et al.,* 1991; Lewis *et al.,* 1991; Abrams *et al.,* 1995), which all produced neutralizing as well as protective responses in pigs. A neutralizing antibody response has been observed in mice immunized with a DNA vaccine expressing empty capsid (Chinsangaram *et al.,* 1998). Cedillo-Barron *et al.* (2001) have reported that co-administration of a plasmid encoding porcine GM-CSF and a empty FMDV capsid (P1-2A) improved the FMDV-specific antibody response. Empty capsids induced in cattle at FMDV-specific neutralizing antibody response and protected them from FMD after direct inoculation challenge in the tongue and contact exposure to an infected animal (Grubman and Mason, 2002). Recently, the precursor capsid proteins (P1-2A) of FMDV serotypes Asia1 (Renji, 2001) and O, A_{22} and C (Balamurugan, 2002), expressed as a secretory proteins in yeast (*Pichia pastoris*), and the expressed P1-2A protein of FMDV srotypes O (Balamurugan *et al.,* 2003) and Asia1, A_{22} and C were found to induce neutralizing antibodies and protective response in guinea pigs in single vaccination (Balamurugan *et al*., 2004).

3.6 Chimeric viral vaccines

Adaptation of field isolates for vaccine purposes is cumbersome, time consuming, and expensive. As an alternative, construction of recombinant FMDVs followed by their use in production of inactivated vaccines is proposed. The advantage of such a strategy could be the possibility of manipulating the antigenicity of these viruses by substituting the antigenic coding regions (i.e., structural proteins) in cDNA clone of a suitable strain. Kitson *et al.* (1991) have constructed poliovirus recombinants with sequences corresponding to FMDV antigenic sites. Viable virus was recovered from these plasmids, in which the VP1 B-C loop (antigenic site 1) of poliovirus type 1 Sabin strain had been replaced with sequences derived from the G-H loop of VP1 (antigenic site 1) or βB-βC loop of VP1 (antigenic site 3) of FMDV type O. The G-H loop of FMDV VP1 (aa 132–155) Is a prominent feature on the virion surface and has an important role in vaccine efficacy and generation of antigenic variants. Using an infectious cDNA of FMDV, Rieder *et al.* (1994) have constructed serotype A viruses in which the βG-βH loop had been substituted with homologous sequences of serotype O or C. These chimeric viruses replicated to high titers and displayed plaque morphology similar to that of wt viruses, demonstrating that the functions provided by the loop can be readily exchanged between serotypes.

Expression of infectious viral RNAs through *in vitro* transcription of cDNA-containing vectors has several advantages. Namely, (i) the infectivity is less dependent on RNA degradation since it presumably occurs only within cells where the RNAs are synthesized, and the replication process can overcome detrimental effects resulting from degradation, (ii) the *in vitro* transcription and RNA transfection is not necessary and this is particularly important for RNA viruses for which the production of good yields of highly infectious full-length transcripts may be problematic, (iv) costly reagents such as cap analogs and RNA polymerases are not required, (v) it renders the expression of viral genes largely independent of the viral replication process. This might be very convenient when studying the role and/or localization of proteins expressed by mutant viral RNAs unable to replicate in cells. These *in vitro* produced viral transcripts would then behave like messenger RNAs produced by a host RNA polymerase, still able to express native or mutant proteins without being replicated (Van Bokhoven *et al.,* 1993). Infectious cDNA has many advantages over inactivated vaccines, namely the possibility of attenuation, high stability, absence of live virus, induction of both humoral and cell-mediated immune response, and absence of problems associated with the existence of pre-immunity. Despite the fact that, considered historically, the first infectious clones of RNA viruses were cDNA clones, to date there are only a few examples of animal viruses for which full-length cDNA containing vectors were successfully used for *in vivo* production of infectious RNA.

Although chimeric vaccines are safe theoretically, they have potential disadvantages. There are chances of recombination leading to production of virulent viruses *in vivo*. A chimeric cDNA clone between serotypes A and SAT 2 was constructed by inserting external capsid coding region of the vaccine strain ZIM/7/83/2 into the backbone of the A 12 cDNA clone (Van Rensburg and Mason, 2002). Preliminary evaluation of the recombinant revealed its slower growth as compared with the parental ZIM/7/83/2, although similar antigen yields could be obtained. The chimera was found to be less thermostable than the parental strain, suggesting its unsuitability for inactivated vaccine production (Van Rensburg and Mason, 2002).

3.7 Genetically engineered attenuated vaccines

Dangers inherent to high potential for variation and adaptation exhibited by FMDV have hampered the use of

classical attenuated strains, obtained by adaptation and further passaging of virulent viruses in insusceptible hosts, as vaccines (Sagedahl *et al.,* 1987). They were due to frequent reversion of attenuated viruses to virulent forms (Cao *et al.,* 1991) as well as to the fact that viral strains attenuated for a given host may be virulent for other hosts (Sagedahl *et al.,* 1987). Chimeric viruses in which the RGD receptor-binding site (McKenna *et al.,* 1995) or L gene (Mason *et al.,* 1997) were deleted induced protection in natural host without producing clinical symptoms. In spite of these promising results, wide FMDV host range and high potential for variation of the virus make a careful study of the stability and pathogenicity of new recombinant vaccines necessary before they can be considered for field trials (Sobrino *et al.,* 2001).

3.8 Recombinant viral vector vaccines

A more efficient induction of protective immunity by live vaccines as compared to inactivated ones has been reported for a number of viruses including picornaviruses (Usherwood and Nash, 1995). This can expected from a requirement for a board spectrum of immune responses for achievement an efficient protection, as observed in animals following natural infection. One strategy to achieve this goal is to present foreign antigens expressed through either replicative form of genome encoded by recombinant viral vectors. The immunogenicity of a recombinant adenovirus and vaccinia virus expressing P1 protein of FMDV (administered either individually or sequentially) was analyzed by Sanz-Parra *et al.* (1999). Double immunization with a recombinant adenovirus elicited an antiviral immune response and protected pigs partially against viral challenge. The authors concluded that the protection resulted from FMDV-specific T cell responses but not antibodies. It is an established fact that introduction of immunogenic domain of VP1 protein alone cannot induce protection even though FMDV neutralizing antibodies are elicited (Brown, 1992; Huang *et al.,* 1999).

A recombinant replication-defective human adenovirus serotype 5 carrying FMDV P1-2A and 3C protease elicited both neutralizing antibodies and protection when challenged with virulent virus in mice (Mayr *et al.,* 1999). A recombinant Vaccinia virus expressing FMDV P1elicited high titer neutralizing antibodies against both homologous FMDV and Vaccinia virus as measured by neutralization assay (Sanz-Parra *et al.,* 1999). Liquid phase blocking ELISA using the whole virus as antigen showed high titer antibodies against homologous FMDV similar to those induced by conventional inactivated vaccine. Vaccinia virus-based vector could be used as a live immunogen delivering system in a mouse model (Berinstein *et al.,* 2000). The use of adenovirus vectors for delivery of protective immunogens from numerous pathogens is being explored as a vaccine strategy for several diseases. Adenovirus targets upper respiratory and gastrointestinal tracts, thereby inducing local mucosal immune responses and triggering cellular immunity. Since initial site of FMDV infection is upper respiratory tract the ability to deliver FMDV nucleic acid to this area would resemble natural infection and could be important in induction of protective immunity (Xiang *et al.,* 1996; Grubman and Mason, 2002).

Currently, one of the most promising vaccine candidates utilizes a viral capsid subunit delivered to animals by using a live virus as vector (Grubman and Mason, 2002). This candidate, a replication-defective recombinant human adenovirus containing the capsid and 3C protease coding regions of FMDV induces an FMDV-specific neutralizing antibody response. Upon challenge with a virulent animal-passaged homologous virus, pigs and cattle vaccinated with the recombinant adenovirus are protected from clinical signs of FMD as well as viral replication. Inoculation of a high dose of this vaccine protected pigs from challenge as early as 7 days after vaccination (Grubman and Mason, 2002). DNA vaccination has proved to be a promising venue for recombinant vaccines. The emergence of gene adjuvants has further added a powerful weapon to the arsenal of DNA vaccines.

3.9 Self-replicating genetic vaccines

A major rationale for putting antigen-coding genes under the control of alphavirus RNA replicase was to enhance antigen expression and presentation. A fundamental difference between replicase-based and conventional DNA vaccines is the fact that viral RNA like intact virus replicates inside the transfected host. Transfection of host cells with replicase-based genetic vaccines could trigger a series of danger signals (Matzinger, 1998). Replicase-based DNA or RNA induces apoptotic death of the host cell *in vitro* just as does alphavirus infection. (Ying *et al.,* 1999). These apoptotic cells may be picked up by dendritic cells for presentation to the immune system (Albert *et al.,* 1998). Transfection with self-replicating genetic vaccines may also cause production of heat shock proteins in transfected or by-stander cells (Chelbi-Alix and Sripati, 1994). The viral replicase has a powerful adjuvant effect due to production of viral double-stranded RNA (dsRNA) intermediates, since ds RNA itself is a potent inducer of IFN and can function as a strong adjuvant in cellular and humoral immune responses. Various molecules are known to bind to and be activated by dsRNA. The best characterized are 2'-5' oligoadenylate (2-5A) synthetase and RNA-dependent protein kinase (PKR). The 2-5A system contributes to the antiviral effect of IFN through the synthesis of 2-5A and its activation of RNase, which degrades both viral and cellular

RNA. PKR expression both induces and is induced by IFN. PKR is then activated by dsRNA to phosphorylate its substrates including e-IF2. This results in the inhibition of translation, further diminishing viral replication. The cellular death observed in response to dsRNA is likely to be mediated by both the 2-5A system-induced RNase as well as substrates of PKR (Rivas *et al.,* 1998). IFN-γ potentiates the apoptotic effects of dsRNA (Tanaka *et al.,* 1998). Thus these vectors have high potential for being regarded as media for the development of new generation vaccines against FMD.

3.10 Transgenic plants with expressed FMDV proteins

Expression of antigens in transgenic plants has been increasingly used as alternative to classical methodologies for antigen expression for the development of experimental vaccines. Foliar extracts of the plants from a Tobacco mosaic virus-based vector carrying complete FMDV VP1 protein showed specific antibody response against VP1 as well as whole virus and elicited a protective response against experimental challenge with virulent FMDV in mice (Wigdorovitz *et al*., 1999a). Wigdorovitz *et al.* (1999b) have reported immunization of mice by parenteral administration of leaf extracts or feeding freshly harvested leaves from a transgenic alfalfa plant. The immunized mice developed virus specific immune response to trans gene (synthetic peptide) as well as intact virus and they were also protected against experimental challenge virus. There is another report about protective immune response in animals to a synthetic peptide mimicking FMDV VP1 protein (aa 135–160) and intact VP1 particles expresse in plants (Carrillo *et al.,* 1998). An epitope from FMDV has been fused to a glucuronidase (gus A) reporter gene allowing selection of transgenic plants by the glucuronidase activity (Carrillo *et al.,* 1998). The FMDV epitope expressed in plants was highly immunogenic in mice, which developed strong antibodies against a synthetic peptide (the VP1 region of aa 135–160), native viral VP1 and purified FMDV. Moreover, the mice were completely protected against experimental challenge with the virulent virus. This constitutes the first report of a peptide-based vaccine produced in transgenic plants that induced a protective immune response in experimental hosts (Dus Santos *et al.,* 2002). Wu *et al.* (2003) have expressed two immunogenic dominant epitopes of FMDV serotype O in tobacco plants using a vector based on recombinant tobacco mosaic virus. One of the immunogenic dominant epitopes made up of 11 amino acids protected pigs from challenge. Also, these results demonstrated the possibility of using a novel and simple methodology for obtaining transgenic plants expressing high levels of foreign proteins, which could be directly applied in the development of plant-based vaccines.

4. Conclusions

Vaccination has proved a powerful defense against a range of infectious diseases of humans and animals. However, its potential to control major epidemics of FMD in livestock is contentious. FMD is a highly contagious disease and is on the A list of infectious diseases of animals of the Office International des Epizooties (OIE) and has been recognized as the most important constraint to international trade in animals and animal products. The only way of control is through vaccination followed by proper monitoring of the animal movement. The conventional vaccines currently used have advantages but they have to be checked on different targets, as the control of FMD becomes one of the urgent needs of the world. The new generation vaccines based on viral proteins, protein fragments and nucleic acids are attractive because of their stability, which is one of important features of a vaccine. Subunit vaccines, synthetic peptide vaccines, DNA vaccines, cytokine-enhanced DNA vaccines, recombinant empty capsid vaccines, chimeric viral vaccines, genetically engineered attenuated vaccines, recombinants viral vector vaccines, self-replicating genetic vaccines and transgenic plants with expressed viral proteins are the present vaccine development strategies for FMD control. The control is a timely need for the countries, which are endemic to FMD. In the next future the use of recombinant vaccines in ensuring effective immune response will apparently become acceptable. Spread of FMD to the disease-free countries and the restrictions to stamping-out policy in certain endemic countries represent a challenge to scientists to search for alternative FMD control strategies.

References

Abrams CC, King AMQ, Belsham GJ (1995): Assembly of foot and mouth disease virus empty capsids synthesised by a vaccinia virus expression system. *J. Gen. Virol.* **76,** 3089–3098.

Acharya R, Fry E, Stuart D, Fox G, Rowlands D, Brown F (1989): Three-dimensional structure of Foot and mouth disease virus at 2.9° resolution. *Nature* **337,** 709–716.

Albert MC, Sauter B, Bhardwaj N (1998): Dendritic cells require antigen from apoptotic cells and induce class I-restricted CTLs. *Nature* **392,** 86–99.

Atkins GJ, Sheahan BJ, Liljestrom P (1999): The molecular pathogenesis of Semliki Forest virus: a model virus made useful. *J. Gen. Virol*. **80,** 2287–2297.

Bachrach HL, Moore DM, McKerche PD, Polatnick J (1975): Immune and antibody responses to an isolated capsid protein of foot and mouth disease virus. *J. Immunol.* **115,** 1636–1641.

Balamurugan (2002): Studies on the production and immuno-genicity of recombinant P1 protein of FMDV type 'O' in

yeast expression system, *Ph.D. Thesis.* Submitted to IVRI, Izatnagar, U.P., India.

Balamurugan V, Renji R, Saha SN, Reddy GR, Gopalakrishna S (2003): Protective immune response of the capsid precursor polypeptide (P1) of foot and mouth disease virus type 'O' produced in *Pichia pastoris*. *Virus Res.* **92,** 141–149.

Balamurugan V, Renji R, Venkatesh G, Reddy GR, Nair SP, Ganesh K, Suryanarayana VVS (2004): Protective immune response against foot and mouth disease virus challenge in guinea pigs vaccinated with recombinant P1 ployprotein expressed in *Pichia pastoris*. *Arch. Virol.* (in press).

Barnett PV, Carabin H (2002): A review of emergency foot and mouth disease (FMD) vaccines. *Vaccine* **20,** 1505–1514.

Barnett PV, Garland AJM, Kitching RP, Schermbrucker CG (2002): Aspects of emergency vaccination against foot and mouth disease. *Comp. Immunol. Microbiol. Infect. Dis.* **25,** 345–364.

Barnett PV, Keel P, Reid S, Armstrong RM, Statham RJ, Voyce C, Aggarwal N, Cox SJ (2004): Evidence that high potency foot and mouth disease vaccine inhibits local virus replication and prevents the 'carrier' state in sheep. *Vaccine* **22,** 1221–1232.

Barteling SJ, Vreeswijk L (1991): Development of foot and mouth disease vaccines. *Vaccine* **9,** 75–88.

Bayry J, Prabhudas K, Gopalakrishna S, Patil PK, Ramakrishna C, Mishra LD, Suryanarayana VVS (1999): Protective immune response to 16kDa immunoreactive recombinant protein encoding the C-terminal VP1 portion of Foot and Mouth Disease Virus type Asia I. *Microbiol. Immunol*. **43,** 768–771.

Beard C, Ward G, Rieder E, Chingsangaram J, Grubman MJ, Mason PW (1999): Development of DNA vaccines for foot and mouth disease, evaluation of vaccines encoding replicating and non-replicating nucleic acids in swine. *J. Biotechnol.* **73,** 243–249.

Beck E, Strohmaier K (1987): Subtyping of European foot and mouth disease virus strains by nucleotide sequence determination. *J. Virol.* **61,** 1621–1629.

Belsham GJ, Abrams CC, King AM, Roosien J, Vlak JM (1991): Myristoylation of foot and mouth disease virus capsid protein precursors is independent of other viral proteins and occurs in both mammlian and insect cells. *J. Gen. Virol.* **72,** 747–751.

Benvenisti L, Rogel A, Kuznetzova L, Bujanover S, Becker Y, Stram Y (2001): Gene gun-mediated DNA vaccination against foot and mouth disease virus. *Vaccine* **19,** 3885–3895.

Berinstein QA, Tami C, Taboga O, Smitsaart E, Carillo E (2000): Protective immunity against Foot and Mouth Disease virus induced by a recombinant vaccinia virus. *Vaccine* **18,** 2231–2238.

Blanco E, Garcia-Briones M, Sanz-Parra A, Gomes P, De Oliveira E, Valero ML, Andreu D, Ley V, Sorbrino F (2001): Identification of T-cell Epitopes in Non-structural Proteins of foot and mouth disease virus. *J. Virol.* **75,** 3164–3174.

Borrego B, Novella SI, Andreu D, Giralt E, Domingo E (1993): Distinct repertoire of antigenic variants of foot and mouth disease virus in the presence and absence of immune selection. *J. Virol.* **67,** 6071–6079.

Brown F (1988): Use of peptides for immunization agaisnt foot and mouth disease. *Vaccine* **6,** 180–183.

Brown F (1992): New approach to vaccination against foot and mouth disease. *Vaccine* **10,** 1022–1026.

Brown F (1999): Foot and mouth disease and beyond: vaccine design, past, present and future. *Arch. Virol.* (Suppl.) **15,** 179–188.

Brown F (2003): The history of research in foot and mouth disease. *Virus Res.* **91,** 3–7.

Cao X, Bergmann IE, Beck E (1991): Comparison of the 5' and 3' untranslated genomic regions of virulent and attenuated foot and mouth disease viruses (strains O1 Campos and C3 Resende). *J. Gen. Virol.* **72,** 2821–2825.

Carrillo C, Wigdorovitz A. Oliveros JC, Zamorano PI, Sasir AM, Gomez N, Salinas J, Escribano JM, Borca MV (1998): Protective immune response against foot and mouth disease virus with VP1 expressed in transgenic plants. *J. Virol.* **72,** 1688–1690.

Cedillo-Barron L, Foster-Cuevas M, Belsham GJ, Lefevre F, Parkhouse RME (2001): Induction of a protective response in swine vaccinated with DNA encoding foot and mouth disease virus empty capsid proteins and the 3D RNA polymerase. *J. Gen. Virol.* **82,** 1713–1724.

Cedillo-Barron L, Foster-Cuevas M, Cook A, Gutierrez-Castaneda B, Kollnberger S, Lefevre F, Parkhouse RME (2003): Immunogenicity of plasmids encoding T and B cell epitopes of foot and mouth disease virus (FMDV) in swine. *Vaccine* **21,** 4261–4269.

Chelbi-Alix MK, Sripati CE (1994): Ability of insulin and DsRNA to induce interferon system and Hsp 70 in fibroblast and epithelial cells in relation to their effects on cell growth. *Exp. Cell Res.* **213,** 383–389.

Chen BJ, Sung WH, Sheih HK (1999): Managing an animal health emergency in Taipei China: Foot and mouth disease. *Rev. Sci. Tech. Off. Int. Epizoot.* **18,** 186–192.

Chinsangaram J, Beard C, Mason PW, Zellner MK, Ward G, Grubman MJ (1998): Antibody Response in Mice Inoculated with DNA Expressing Foot and Mouth Disease Virus Capsid Proteins. *J. Virol*. **72,** 4454–4457.

Chinsangaram J, Moraes MP, Koster M, Grubman MJ (2003): Novel viral disease control strategy: adenovirus expressing alpha interferon rapidly protects swine from foot and mouth disease. *J. Virol.* **77,** 1621–1625.

Collen T (1994): Foot and mouth disease virus (aphthovirus): Viral T cell epitopes. In Goddeevis, BML, Morrison I (Eds): *Cell Mediated Immunity in Ruminants*. CRC press, Inc, Boca Raton, pp. 173–197.

Collen T, DiMarchi R, Doel TR (1991): A T cell epitope in VP1 of foot and mouth disease virus is immunodominant for vaccinated cattle. *J. Immunol.* **146,** 749–755.

Costa-Pereira AP, Williams TM, Strobi B, Watling D, Briscoe J, Kerr IM (2002): The antiviral response to gamma interferon. *J. Virol.* **76,** 9060–9068.

Cox SJ, Barnett PV, Dani P, Salt JS (1999): Emergency vaccination of sheep against foot and mouth disease: protection

against disease and reduction in contact transmission. *Vaccine* **17,** 1858–1868.

David M, Torres A, Mebus C, Carrillo BJ, Schudel A, Fondevilla N, Balnco Viera J, Marcovecchio EE (1993): Further studies on foot and mouth disease virus in the Llama. *Proc. Ann. Meet. U. S. Anim. Hlth. Assoc.* **97,** 280–285.

Domingo E, Davila M, Ortin J (1980): Nucleotide sequence heterogeneity of the RNA from a natural population of foot and mouth virus. *Gene* **11,** 333–346.

Domingo E, Diez J, Martinez MA, Hernandez JH, Holguin A, Borrego B, Mateu MG (1993): New observations on antigenic diversifications of RNA viruses. Antigenic variation is not dependent on immune selection. *J. Gen. Virol.* **74,** 2039–2045.

Domingo E, Escarmis C, Baranowski E, Ruiz-Jarabo CM, Carrillo E, Nunez JI, Sobrino F (2003): Evolution of foot and mouth disease virus. *Virus Res.* **91,** 47–63.

Domingo E, Holland JJ (1998): High error rates, population equilibrium and evolution of RNA replication systems. In Domingo E, Holland JJ, Ahiquist P (Eds): *RNA genetics, Variability of RNA Genomes*. Vol III, CRC Press, Boca Raton, Fla, pp. 3–36.

Domingo E, Martinez-salas E, Sobrino F, de la torre JC, Portela A, Ortin J, Lopez-galindez C, Perez-Brena P, Villanuera N, Nareja R, Vande Pol S, Steinhaeur D, De Polo N, Holland JJ (1985): The quasispecies (extremely heterogenous) nature of viral RNA populations: Biological relevance. (A review). *Gene* **40,** 1–8.

Domingo E, Mateu MG, Martinez MA, Dopazo J, Moya A, Sobrino F (1990): Genetic variability and antigenic diversity of Foot and Mouth Disease Virus. In Kurstak, E, Marusyk RG, Murphy, FA, van Ragenmortel MHV (Eds): *Applied Virology Research, Virus Variability, Epidemiology and Control*. Vol 2. Plenum Press, New York, pp. 233–266.

Drake JW, Holland JJ (1999): Mutation rates among RNA viruses. *Proc. Natl. Acad. Sci. USA* **96,** 13910–13913.

Dunham SP (2002): The application of nucleic acid based vaccines in veterinary medicine. *Res. Vet. Sci.* **73,** 9–16.

Dus Santos MJ, Wigdorovitz A, Trono K, Rios RD, Franzone PM, Gil F, Moreno J, Carrillo C, Escribano JM, Borca MV (2002): A novel methodology to develop a foot and mouth disease virus (FMDV) peptide-based vaccine in transgenic plants. *Vaccine* **20,** 1141–1147.

Francis MJ, Hastings GZ, Syred AD, McGinn B, Brown F, Rowlands DJ (1987): Non-responsiveness to a foot and mouth disease virus peptide overcome by addition of foreign helper T-cell determinants. *Nature* **330,** 168–170.

Garland AJM (1997): The availability of foot and mouth disease vaccine for emergency vaccination in Europe. *Thirty Second Meeting of the European Commission for the control of Foot and Mouth Disease*, Rome, Italy, 2–4 April, FAO, Rome.

Gebauer F, de la Torre JC, Gomes I, Mateu MG, Barahona H, Tiraboschi B, Bergmann I, Auge de Mello P, Domingo E (1988): Rapid selection of genetic and antigenic variants of foot and mouth disease virus during persistence in cattle. *J. Virol.* **62,** 2041–2049.

Grubman MJ, Baxt B (2004): Foot and Mouth Disease. *Clin. Microbiol. Rev.* **17,** 465–493.

Grubman MJ, Lewis SA, Morgan DO (1993): Protection of swine against foot and mouth disease with viral capsid proteins expressed in heterologous systems. *Vaccine* **11,** 825–829.

Grubman MJ, Mason PW (2002): Prospects, including time frames, for improved foot and mouth disease vaccines. *Rev. Sci. Tech. Off. Int. Epizoot.* **21,** 589–600.

Grubman MJ, Morgan DO, Kendall J, Baxt B (1985): Capsid intermediates assembled in a foot and mouth disease virus genome RNA-programmed cell-free translation system and in infected cells. *J. Virol.* **56,** 120–126.

Huang H, Yang Z. Xu Q, Sheng Z, Xie Y, Yan W, You Y, Sun L, Zheng Z (1999): Recombinant fusion protein and DNA vaccines against foot and mouth disease virus infection in guinea pig and swine. *Viral Immunol.* **12,** 1–8.

Kim JJ, Yang JS, Manson KH, Weiner DB (2001): Modulation of antigen-specific cellular immune responses to DNA vaccination in rhesus macaques through the use of IL-2, IFN-gamma, or IL-4 gene adjuvants. *Vaccine* **19**, 2496–2505.

Kitching RP (1998): A recent history of foot and mouth disease virus. *J. Comp. Pathol.* **118,** 89–108.

Kitching RP (2002a): Clinical variation in foot and mouth disease: cattle. *Rev. Sci. Tech. Off. Int. Epizoot.* **21,** 499–504.

Kitching RP (2002b): Future research on foot and mouth disease. *Rev. Sci. Tech. Off. Int. Epizoot.* **21,** 885–889.

Kitching RP (2002c): Identification of foot and mouth disease virus carrier and subclinically infected animals and differentiation from vaccinated animals. *Rev. Sci. Tech. Off. Int. Epizoot.* **21,** 531–538.

Kitching RP, Hughes GJ (2002): Clinical variation in foot and mouth disease: sheep and goats. *Rev. Sci. Tech. Off. Int. Epizoot.* **21,** 505–512.

Kitching RP, Rendle R, Ferris NP (1988): Rapid correlation between the field isolates and vaccine strains of foot and mouth disease virus. *Vaccine* **6**, 403–408.

Kitching RP, Salt JS (1995): The interference by maternally-derived antibody with active immunization of farm animals against foot and mouth disease. *Br. Vet. J.* **151,** 379–389.

Kitson JDA, Burke KL, Pullen LA, Belsham GJ, Almond JW (1991): Chimeric polioviruses that include sequences derived from two independent antigenic sites of foot and mouth disease virus (FMDV) induce neutralizing antibodies against FMDV in Guinea pigs. *J. Virol.* **65,** 3068–3075.

Kleid DG, Yansura D, Small B, Dowbenko D, Moore DM, Grubman MJ, McKerecher PD, Morgan DO, Robertson BH, Bachrach HL (1981): Cloned viral protein vaccine for foot and mouth disease: responses in cattle and swine. *Science* **214,** 1125–1129.

Knowles NJ, Samuel AR (2003): Molecular epidemiology of foot and mouth disease virus. *Virus Res.* **91,** 65–80.

Lewis SA, Morgan DO, Grubman MJ (1991): Expression, processing and assembly of foot and mouth-disease virus capsid structures in heterologous system. Induction of a neutralizing antibody response in guinea pigs. *J. Virol.* **65,** 6572–6580.

Loeffler F, Frosch P (1898): Summarischer Bericht uber die Ergebnisse der Untersuchungen der Kommoission zur Erforchung der Maul-und-Klanenseuche. *Zentbl. Bacteriol. Parasitenk. Infektkrankh.* **23,** 257–259.

Manoj Kumar R, Sanyal A, Tosh C, Hemadri D, Mohapatra JK, Venkataramanan R, Bandyopadhyay SK (2003): Mutations in the 1D gene (VP1) of Foot and mouth disease virus serotype Asia1 during serial cytolytic infections in cell culture. *Arch. Virol.* **148,** 1815–1825.

Mason PW, Piccone ME, McKenna TS, Chinsangaram J, Grubman MJ (1997): Evaluation of a live-attenuated foot and mouth disease virus as a vaccine candidate. *Virology* **227,** 96–102.

Mateu MG (1995): Antibody recognition of picornaviruses and escape from neutralization, A structural review. *Virus Res.* **38,** 1–24.

Mateu MG, da Silva JL, Rocha M, de Brum DL, Alonso A, Enjuanes L, Domingo E, Barahona H (1988): Extensive antigenic heterogeneity of foot and mouth disease virus of serotype C. *Virology* **167,** 113–124.

Mateu MG, Hernandez J, Martinez MA (1994): Antigenic heterogeneity of foot and mouth disease virus serotype in the field is mediated by very limited sequence variation at several antigenic sites. *J. Virol.* **68,** 1407–1417.

Matzinger P (1998): An innate sense of danger. *Semin. Immunol*. **10,** 339–415.

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

McKenna TSC, Luborth J, Rieder E, Baxt B, Mason PW (1995): Receptor binding site-deleted foot and mouth disease (FMD) virus protects cattle from FMD. *J. Virol.* **69,** 5787–5790.

Meloen RH, Barteling SJ, Niepmann M, Beck E (1986): An epitope located at the C-terminus of isolated VP1 of foot and mouth disease virus O induces neutralizing activity but poor protection. *J. Gen. Virol.* **67,** 289–294.

Meloen RH, Casal JI, Dalagaard K, Langeveld JP (1995): Synthetic peptide vaccines: success at last. *Vaccine* **13,** 885–886.

Racaniello VR (2001): Picornaviridae: The viruses and their replication. In Knipe DM, Howley PM, Griffin DE, Lamb RA, Martin MA, Roizman B, Straus SE (Eds): *Field Virology*. Vol. 1, Lippincott Williams and Wilkins, pp. 685–722.

Renji (2001): Production of P1 poly protein of Foot and Mouth Disease virus in yeast cells and Studies on its application. *Ph.D. Thesis*. Submitted to IVRI. Deemed University, Izatnagar, U.P., India.

Rieder E, Baxt B, Lubroth J, Mason PW (1994): Vaccines prepared from Chimeras of foot and mouth Disease virus (FMDV) induce neutralizing antibodies and protective immunity to multiple serotypes of FMDV. *J. Virol.* **68,** 7092–7098.

Rigden RC, Carrasco CP, Barnett PV, Summerfield A, McCullough KC (2003): Innate Immune responses following emergency vaccination against foot and mouth disease virus in pigs. *Vaccine* **21,** 1466–1477.

Rivas C, Gil J, Melkova Z, Esteban M, Diaz-Guerra M (1998): Vaccinia virus E3L protein is an inhibitor of the interferon (i.f.n.)-induced 2-5A synthetase enzyme. *Virology* **243,** 406–414.

Robinson HL, Hunt LA, Webster RG (1993): Protection against a lethal influenza virus challenge by immunization with a haemagglutination-expressing plasmid DNA. *Vaccine* **11,** 957–960.

Roosien J, Belsham GJ, Ryan MD, King AMQ, Black JM (1990): Synthesis of foot and mouth disease virus capsid proteins in insect cells using baculovirus expression vectors. *J. Gen. Virol.* **71,** 1703–1711.

Rowlands DJ (1994): Experimental determination of immunogenic sites. In Nicholson BH (Ed.): *Synthetic Vaccines*. Blackwell Scientific Publications, Oxford, pp. 137–168.

Rowlands DJ, Sanger DV, Brown F (1975): A comparative chemical and serological study of full and empty particles of foot and disease virus. *J. Gen. Virol.* **26,** 227–238.

Rweyemamu MM, Terry G, Pay TWF (1979): Stability and immunogenicity of empty particles of foot and mouth disease virus. *Arch. Virol*. **59,** 69–79.

Sagedahl A, Giraudo AT, Augo de Mello P, Bergmann IE, La Torre JL, Scodeller EA (1987): Biochemical characterization of an aphthovirus type C3 strain Resende attenuated for cattle by serial passages in chicken embryos. *Virology* **157,** 366–374.

Salt JS (1993): The carrier state in foot and mouth disease virus. An immunological review. *Br. Vet. J.* **149,** 207–223.

Salt JS, Barnett PV, Dani P, Williams L (1998): Emergency vaccination of pigs against foot and mouth disease: protection against disease and reduction in contact transmission. *Vaccine* **16,** 746–754.

Salt JS, Mulcahy G, Kitching RP (1996): Isotype-specific antibody responses to foot and mouth disease virus in sera and secretions of 'carrier' and 'non-carrier' cattle. *Epidemiol. Infect.* **117,** 349–360.

Sangar DV, Black DN, Rowlands DJ, Brown F (1977): Biochemical mapping of the foot and mouth disease virus genome. *J. Gen. virol*. **35,** 281–289.

Sanz-Parra A, Jimenez-Clavero MA, Garcia-Briones MM, Blanco E, Sobrino F, Ley Y (1999): Recombinant viruses expressing the foot and mouth disease virus capsid precursor polypeptide (P1) induce cellular but not humoral antiviral immunity and partial protection in pigs. *Virology* **259,** 129–134.

Sasaki S, Inamura K, Kenji O (1999): Genes That Induce Immunity-DNA Vaccines. *Microbiol. Immunol.* **43,** 191–200.

Scudamore JM, Harris DM (2002): Control of foot and mouth disease: lessons from the experience of the outbreak in Great Britain in 2001. *Rev. Sci. Tech. Off. Int. Epizoot.* **21,** 699–709.

Shieh JJ, Liang CM, Chen CY, Lee F, Jong MH, Lai SS, Liang SM (2001): Enhancement of the immunity to foot and mouth disease virus by DNA priming and protein boosting immunization. *Vaccine* **19,** 4002–4010.

Sobrino F, Blanco E, Garcio-Briones M, Ley V (1999): Synthetic peptide vaccines: foot and mouth disease virus as a model. *Dev. Biol. Stand.* **101,** 39–43.

Sobrino F, Saiz M, Jimenez-Clavero MA, Nunez JI, Rosas MF, Baranowski E, Ley V (2001): Foot and mouth disease virus: a long known virus, but a current threat. *Vet. Res.* **32,** 1–30.

Strohmaier K, Franze R, Adam KH (1982): Location and characterisation of the antigenic portion of the FMDV immunizing protein. *J. Gen. Virol.* **59,** 295–306.

Suryanarayana VVS, Rao BU, Padayatty JD (1985): Cloning and expression cDNA for the major antigen of foot and mouth disease virus Asia-1 63/72. *Curr. Sci.* **54,** 1044–1048.

Suryanarayana VVS, Venkataraman R, Padayatty JD (1992): Characterization and immune response of a protein produced by a cDNA clone of foot and mouth disease virus type Asia-1 63/72. *Biochem. Inter.* **26,** 1003–1016.

Suttmoller P, Gaggero A (1965): Foot and mouth disease carriers. *Vet. Rec.* **77,** 968–969.

Taboga O, Tami C, Carrillo E, Nunez JI, Rodriguez A, Salt JC, Blanco E, Valero ML, Roig X, Camerero JA, Andreu D, Mateu MG, Girlat E, Domingo E, Sobrino F, Palma EL (1997): A large-scale evaluation of peptide vaccines against foot and mouth disease: lack of solid protection in cattle and isolation of escape mutants. *J. Virol.* **71,** 2606–2614.

Tanaka N, Sato M, Lamphier MS, Nozawa H, Oda E, Noguchi S, Schreiber RD, Tsujimoto Y, Taniguchi T (1998): Type I interferons are essential mediators of apoptotic death in virally infected cells. *Genes Cells* **3,** 29–37.

Tosh C, Hemadri D, Sanyal A, Bandyopadhyay SK (2003): Genetic and antigenic analysis of two recently circulating genotypes of type A foot and mouth disease virus in India: evidence for positive selection in the capsid-coding genes. *Arch. Virol.* **148,** 853–869.

Ulmer JB, Donnhly JJ, Parker Se, Rhodes GH, Felgner PL, Dwarki VJ, Gromkowski SH, Deck RR, DeWitt CM, Friedman A, Hawe LA, Leander KR, Martinez D, Perry HC, Shiver JW, Montgomery DL, Liu MA (1993): Heterologous protection against influenza by injection of DNA encoding a viral protein. *Science* **259,** 1745–1749.

Usherwood EJ, Nash AA (1995): Lymphocyte recognition of picorna viruses. *J. Gen. Virol.* **76,** 499–508.

Van Bokhoven H, Verver J, Wellink J, Van Kammen A (1993): Protoplasts transiently expressing the 200K coding sequence of cowpea mosaic virus B-RNA support replication of M-RNA. *J. Gen. Virol*. **74,** 2233–2241.

Van Rensburg HG, Mason PW (2002): Construction and evaluation of a recombinant foot and mouth disease virus: implications for inactivated vaccine production. *Ann. New York Acad. Sci.* **969,** 83–87.

Volpina OM, Surovoy AY, Zhmak MN, Kuprianova MA, Koroev DO, Chepurkin AV, Toloknov AS, Ivanov VT (1999): A peptide construct containing B-cell and T-cell epitopes from the foot and mouth disease viral VP1 protein induces efficient antiviral protection. *Vaccine* **17,** 577–584.

Wang CY, Chang TY, Walfield AM, Ye J, Shen M, Chen SP, Li MC, Lin YL, Jong MH, Yang PC, Chyr N, Kramer E, Brown F (2002): Effective synthetic peptide vaccine for foot and mouth disease in swine. *Vaccine* **20,** 2603–2610.

Wang CY, Chang TY, Walfield AM, Ye J, Shen M, Zhang ML, Lubroth J, Chen SP, Li MC, Lin YL, Jong MH, Yang PC, Chyr N, Kramer E, Brown F (2001): Synthetic peptide-based vaccine and diagnostic system for the effective control of FMD. *Biologicals* **29,** 221–228.

Wang JH, Liang CM, Peng JM, Shieh JJ, Jong MH, Lin YL, Sieber M, Liang SM (2003): Induction of immunity in swine by purified recombinant VP1 of foot-and-mouth disease virus. *Vaccine* **21,** 3721–3729.

Ward G, Rieder E, Mason PW (1997): Plasmid DNA encoding replicating foot and mouth disease virus genomes induces antiviral immune responses in swine. *J. Virol.* **71,** 7442–7447.

Wigdorovitz A, Carrillo C, Dus Santos MJ, Trono K, Peralta A, Gomez MC, Rios RD, Franzone PM, Sadir AM, Escribano JM, Borca MV (1999a): Induction of protective antibody response to Foot and Mouth Disease virus in mice following oral or parentral immunization with Alfalfa transgenic plants expressing the viral structural protein VP1. *Virology* **255,** 347–353.

Wigdorovitz A, Perez FDM, Robertson N, Carrillo C, Sadir AM, Morris TJ, Borca MV (1999b): Protection of mice against challenge with Foot and Mouth Disease (FMDV) by immunization with foliar extracts from plants infected with recombinant Tobacco mosaic Virus expressing the FMDV structural protein VP1. *Virology* **264,** 85–91.

Wolff JA, Malone RW, Williams P, Chong W, Acsadi G, Jani A, Felger PL (1990): Direct gene transfer into mouse muscle in vivo. *Science* **247,** 1465–1468.

Wong HT, Cheng SC, Chan EW, Sheng ZT, Yan WY, Zheng ZX, Xie Y (2000): Plasmids encoding foot and mouth disease virus VP1 epitopes elicited immune responses in mice and swine and protected swine against viral infection. *Virology* **278,** 27–35.

Wong HT, Cheng SC, Sin FW, Chan EW, Sheng ZT, Xie Y (2002): A DNA vaccine against foot and mouth disease elicits an immune response in swine, which is enhanced by co-administration with interleukin-2. *Vaccine* **20,** 2641–2647.

Wu L, Jiang L, Zhou Z, Fan J, zhang Q, Zhu H, Han Q, Xu Z (2003): Expression of foot and mouth disease virus epitopes in tobacco by a tobacco mosaic virus-based vector. *Vaccine* **21,** 4390–4398.

Xiang ZQ, Yang Y, Wilson JM, Rrtl HC (1996): A replication-defective human adenovirus recombinant serves as a highly efficacious vaccine carrier. *Virology* **219,** 220–227.

Ying H, Zaks TZ, Wang RF, Irvince KR, Kammula US, Marincola FM, Leitner WW, Restifo NP (1999): Cancer therapy using a self-replicating RNA vaccine. *Nat. Med.* **5,** 823–827.

Zhang HY, Sun SH, Guo YJ, Zhou FJ, Chen ZH, Lin Y, Shi K (2003a): Immune response in mice inoculated with plasmid DNAs containing multiple-epitopes of foot and mouth disease virus. *Vaccine* **21,** 4704–4707.

Zhang Q, Yang YQ, Zhang ZY, Li L, Yan WY, Jiang WJ, Xin AG, Lei CX, Zheng ZX (2002b): Immunogenicity of a recombinant fusion protein of tandem repeat epitopes of

foot and mouth disease virus type Asia 1 for guinea pigs. *Acta Virol.* **46,**1–9.

Zhang Q, Zhu MW, Yang YQ, Shao M, Zhang ZY, Lan HY, Yan WY, Wu JJ, Zheng ZX (2003b): A recombinant fusion protein and DNA vaccines against foot and mouth disease virus type Asia 1 infection in guinea pigs. *Acta Virol.* **47,** 237–243.

Zhang ZD, Hutching G, Kitching P, Alexandersen S (2002a): The effects of gamma interferon on replication of foot and mouth disease virus in persistently infected bovine cells. *Arch. Virol.* **147,** 2157–2167.

Zhang ZD, Kitching RP (2001): The localization of persistent foot and mouth disease virus in the epithelial cells of the soft palate and pharynx. *J. Comp. Pathol.* **124,** 89–94.