Natural and vaccine-induced immunity to foot and mouth disease: the prospects for improved vaccines

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Summary: The review considers both the immune responses of livestock to foot and mouth disease (FMD) virus after infection or vaccination, and the characteristics and properties of FMD viruses and vaccines which are relevant to protection. Particular attention is given to possible approaches which could be used to improve conventional vaccines or produce novel vaccines against this most important disease.

KEYWORDS: Aphthovirus - Foot and mouth disease - Foot and mouth disease vaccines - Immune response - Immunity - Natural immunity - Novel vaccines.

INTRODUCTION

The international impact of foot and mouth disease (FMD) on the productivity of livestock and trade in animals and animal products has given rise to a substantial body of work, dating back to the beginning of the 20th century, on immunity to the disease and the development and efficacy of inactivated viral vaccines. As a result, commercial vaccines are now widely used throughout those parts of the world where FMD is prevalent and examples of their pivotal role in disease eradication can be seen in Western Europe and, most recently, Uruguay. Intensive annual vaccination campaigns, such as those employed in Europe until 1990 to 1991, have the secondary benefit of reducing the probability of spread to neighbouring countries or regions where vaccination is not practised (43).

It is understandable that the achievements made with FMD vaccines have been overlooked in more recent times, in contrast to the disadvantages of their use. In a frequently cited paper, Beck and Strohmaier attributed many of the more recent outbreaks of the A and O serotypes in Western Europe to incompletely inactivated vaccines or escape of virus from laboratories or production units (10). While these general conclusions may be valid, it is interesting to note that the number of outbreaks of the C serotype was negligible during the period covered by this study, despite the widespread production and use of this virus in European trivalent vaccines. A similar situation exists in South America where most outbreaks are attributed to the A and O serotypes, although C serotype vaccine is widely used. It is also perhaps worth reflecting that, since the removal of vaccine cover from Western Europe, ‘natural’ outbreaks have occurred with more or less the same frequency as before but without

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the additional safeguard of immunity in the susceptible livestock population (in Italy and Bulgaria in 1993 and in Greece in 1994 and 1996). The limitations of conventional FMD vaccines have also been highlighted by some of the more recent publications, including several by the author, which have indicated the need for superior FMD products made either synthetically or by recombinant deoxyribonucleic acid (DNA) technology. In some cases, the emphasis has been unreasonable and has diminished the credibility of perfectly acceptable vaccines, while promoting novel formulations which are unlikely to make a significant impact on disease control for many years to come. Some authors have also failed to recognise the well-known limitations of inactivated vaccines and the special characteristics of FMD which complicate the development and use of improved FMD vaccines.

The highly contagious nature of FMD, its host range and the ease with which the disease spreads both nationally and internationally (41) make it vitally important that national disease control authorities continue to employ good quality preparations of conventional FMD vaccines until more effective materials become available. A significant reduction in the use of vaccines in many FMD-endemic parts of the world would have disastrous consequences for the national and international status of the disease.

In this review, the author examines the present state of knowledge on FMD vaccines, with particular respect to immune responses, and attempts to assess some of the characteristics and properties of FMD vaccines in an objective manner, with reference to the extent to which any limitations can be circumvented by modifications to present formulations or development of novel vaccines. The review also deals with the quality of immunity following infection with FMD and subsequent implications for vaccine development.

Infection-induced immunity to foot and mouth disease

Cattle which have recovered from infection with one of the seven serotypes of FMD are not immune to the other serotypes but remain protected against the first serotype for a considerable period of time. However, further rounds of infection with other serotypes may result in less severe clinical responses or protection. Thus, in a study by Cottral and Gailiunas, one animal was completely resistant to infection by a fourth serotype, two animals were completely resistant to a fifth serotype and one animal was completely resistant to a seventh serotype (23). The authors reported that the cross-neutralisation titres were consistent with the protection observed. High-quality immunity, as conferred by a potent vaccine or recent infection (six months post infection [pi]), appears capable of preventing development of any clinical signs of disease, regardless of whether the subsequent challenge is made by injection of virus or contact with infected animals (T.R. Doel, unpublished findings). When convalescent animals were challenged approximately one year after exposure to virus, they were found to be protected, although lesions developed at the inoculation sites (28). In fact, neutralising antibody and protection may persist for the effective lifetime of many cattle, as demonstrated with eight animals which were challenged 5.5 years after initial infection (50). Similar data were reported from another laboratory, in which cattle were held for 4.5 years after initial infection (28). Following challenge, one of three animals was protected. In the absence of more detailed experimentation, it is probable that the degree of protection after a long convalescent period will depend on the serotype, and possibly subtype, of virus used in the experiment (i.e. on the relative
virulence of this strain for cattle), and on variations in the rate of decline of antibody for individual animals. Although the above experiments involved challenge as well as serology, it is perhaps worth mentioning that the correlations which exist between FMD virus (FMDV) specific antibody titres and protection have been made with sera taken from animals shortly after vaccination (usually 21 to 28 days), and it is unreasonable to assume that the antibodies in long-term convalescent animals would be qualitatively equivalent.

Protection is mediated by antibody, as indicated by early passive immunity experiments with cattle and other species (6). This has led to the establishment and general acceptance of correlations between in vitro assays, such as virus-neutralising antibody tests, and protection of cattle, most of the data having been derived from vaccine potency trials (31). While such correlations are and have been of undoubted value, it is worth reflecting on the many variables which are known to, or are believed to, influence the immune response to FMDV (Table I). Not surprisingly, problem sera, in which protection is predicted from an in vitro assay but is not observed (and vice versa), occur quite frequently. Under these circumstances, a passive immunity test in suckling mice may be used as a more accurate in vitro assessment of the protective capacity of a serum (54). Interestingly, a neutralisation test in mice was also found to be deficient in assessing the protective capacity of problem sera (24). The essential difference between the two methods is that, in the passive immunity test, the serum is injected one hour before virus challenge, whereas a virus/serum mixture is made and pre-incubated before injection in the neutralisation test.

Serum immunoglobulin M (IgM) may be detected 3 to 5 days after infection, reaching a peak between 5 to 10 days. Serum IgG1, and IgG2, appear from 4 days onwards and reach maximum levels between 15 to 20 days (1, 24). As stated above, virus-neutralising antibody titres may persist for many years, albeit at reduced titres compared to the peak responses. Although IgM does not generally persist, it has been detected as late as six months post infection in swine (24).

Mucosal immune responses have also been studied following infection of cattle. A peak of neutralising activity attributed to IgM and IgA was observed in the pharyngeal fluid seven days after virus exposure. The peak appeared to be the result of serum and tissue fluid leakage during the inflammatory phase of the infection (48). Between 20 to 60 days post infection (dpi), the neutralising activity of the pharyngeal fluid was attributed exclusively to IgA produced at mucosal surfaces rather than by serum transudation (48). McVicar and Sutmoller (73) reported that the virus-neutralising activities of both serum and oesophageal-pharyngeal fluid were higher in virus-carrier animals than in non-carriers, suggesting the continued stimulation of both compartments of the immune response by the persistent infection. Despite these and other studies, it is difficult to analyse the contribution made by mucosal immunity in the protection of cattle against FMD. This is because mucosal neutralising antibody titres, when present, are accompanied by substantially higher titres in the serum. Thus, serum antibodies will not only protect against generalisation but will also probably reach mucosal surfaces due to tissue damage during the acute phase of the infection, unless mucosal antibody titres are high enough to prevent early virus replication.

The involvement of T-cells in the immune response of cattle to FMDV has only been investigated in recent years and, for the most part, studies have focused on animals which have been vaccinated with inactivated virus or synthetic peptides. Although Borca et al. have proposed that FMDV is a T-independent antigen (14), most of the published work is indicative of T-dependency (18, 19), and has concentrated on
### Table I

*Probable and known variables which influence the immune response to foot and mouth disease virus and vaccine*

<table>
<thead>
<tr>
<th>Stimulus variables</th>
<th>Response variables</th>
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<tbody>
<tr>
<td><strong>Host</strong></td>
<td>Antibodies</td>
</tr>
<tr>
<td>Species</td>
<td>Specificity (number/relative proportions of antigenic sites recognised)</td>
</tr>
<tr>
<td>Breed</td>
<td>Affinity and avidity</td>
</tr>
<tr>
<td>Individuality (particularly MHC)</td>
<td>Isotype (including recognition by F&lt;br&gt; receptor bearing cells)</td>
</tr>
<tr>
<td>Age</td>
<td>Half-lives</td>
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<tr>
<td>Health (e.g. concurrent infections)</td>
<td>Synergy or competition between different antibodies</td>
</tr>
<tr>
<td>Physiological state (e.g. pregnancy, lactation)</td>
<td>Titre and distribution</td>
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<tr>
<td>Other stress factors (e.g. husbandry, climate)</td>
<td></td>
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<tr>
<td>FMD immune status (e.g. maternal antibody)</td>
<td></td>
</tr>
<tr>
<td><strong>Virus</strong></td>
<td>Cells (including memory)</td>
</tr>
<tr>
<td>Dose</td>
<td>Density and number</td>
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<tr>
<td>Route</td>
<td>Distribution/tropism</td>
</tr>
<tr>
<td>Volume</td>
<td>Type (B-cells, T-cells, phagocytes)</td>
</tr>
<tr>
<td>Purity (content and nature of extraneous proteins)</td>
<td>Specificity (number/relative proportions of antigenic sites recognised)</td>
</tr>
<tr>
<td>Virus strain (e.g. physical and antigenic characteristics)</td>
<td>Relative proportions of different cells</td>
</tr>
<tr>
<td>Adjuvant(s)</td>
<td>Half-lives</td>
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</tbody>
</table>

This Table is not intended to be exhaustive and many of the variables are interrelated, e.g. isotype and distribution of antibody.

MHC: major histocompatibility complex

FMD: foot and mouth disease

F<sub>c</sub>: fragment crystallisable

T-cell help and the identification of T-cell epitopes within viral antigens. Having said this, the belief that T-cell help is crucial for the development of an effective and prolonged immune response to FMDV is based more on general immunological principles than on specific scientific proof. In two separate studies on infected cattle, only low-to-moderate *in vitro* proliferation of peripheral blood T-cells was observed (17, 49); similar, in fact, to the T-cell responses of vaccinated animals. This was despite the development of high levels of neutralising antibody in all animals, and complete protection, including the inoculated tongues (T.R. Doel, unpublished findings) of three cattle which were challenged six months after recovery from infection (49). Some of this work was done with major histocompatibility complex (MHC) class II typed animals and there was no evidence of MHC-restricted T-cell responses to whole virus (17). Another interesting feature of these experiments was the biphasic nature of both the neutralising antibody and the T-cell proliferative responses,
in which a coincident peak could be observed at 14 dpi, followed by a trough at approximately 28 dpi, and a gradual increase in both parameters to the end of the experiment at 56 dpi. It is tempting to attribute the recovery of the antibody and T-cell responses after 28 dpi to the establishment of the carrier state and the presence of persistent virus. Although the in vitro proliferative responses of peripheral blood T-cells of once-infected cattle are generally low, a second in vivo challenge enhances the T-cell responses significantly (49). One possible explanation for the apparently poor in vitro T-cell responses following infection is that the circulation of FMDV-specific, active T-cells is limited because of tissue tropism, and the majority are sequestered in the lymph nodes or other tissues of the lymphoreticular system. No information is available on the duration of T-cell responses in FMDV-infected cattle.

In addition to T-helper cell activity with FMDV, immediate and delayed-type hypersensitivity reactions have been observed with guinea-pigs and cattle (63, 93) but no correlation was apparent between the responses in guinea-pigs and protection against challenge. There is no evidence of a role for cytolytic T-cells in the immune response to FMD.

Because of its implications for the quality and duration of the immune response, it is not possible to review aspects of natural immunity to FMD without considering the important phenomenon of persistence. After infection with FMDV, cattle often develop the carrier state, in which infectious virus can be isolated from oesopharyngeal specimens for up to 2.5 years (58, 92). During the period of persistence, there is a gradual decline in the titre and frequency of virus recovery, and intervals as long as 100 days between successful isolations have been reported (11, 92). In addition, a particularly sensitive test, enzyme-linked immunoelectrotransfer blot (EITB), in which serum antibodies against nitro-cellulose blotted non-structural proteins of the virus are detected immunoenzymatically, indicates that FMD may persist beyond the last time point at which infectivity in oesopharyngeal specimens can be detected (11). The non-structural proteins used in the EITB test are 2C, 3A, 3B, 3ABC and 3D (viral ribonucleic acid [RNA] polymerase) and were prepared from recombinant Escherichia coli. Finally, while most persistently infected cattle have significant titres of neutralising antibodies in their sera, seronegative animals are occasionally encountered (97).

Despite the importance of sheep in the epidemiology of FMD, very little is known of their immune response to infection. Virus-neutralising antibodies appear 60 hours post inoculation, reaching a maximum titre at approximately 10 dpi. Titres decrease slightly after day 10 but generally remain at a plateau for at least 147 days (29). The carrier state may develop in sheep and goats, and virus can be recovered in oesopharyngeal specimens from one to nine months after the initial infection in sheep, and more than one month after infection in goats (21). Given the potential importance of sheep in the spread of FMD, and the high sensitivity of the EITB test, it would be worthwhile to re-evaluate the duration of persistence in this species.

The immune response of pigs to infection by FMD has been studied by a number of workers. According to Cunliffe (27), virus-neutralising antibody titres initially rose to peak levels between 7 to 10 dpi and decreased thereafter twelve-fold, until they reached a relatively stable plateau at 28 days, which lasted until the end of the experiment (128 dpi). When five convalescent pigs were challenged at 128 dpi by contact with an infected control animal, only one developed the disease. These results contrast with those of other workers (53, 70), in which approximately 50% of the
animals succumbed to challenge three to six months after the first exposure to FMDV. It would seem, therefore, that the duration of immunity in convalescent pigs is significantly shorter than in cattle. This may owe much to the most important observation that pigs do not become persistently infected. The mucosal immune response of pigs also shows an interesting difference from that of cattle. The neutralising antibody titres of the nasal fluid of pigs are very similar to those found in serum throughout a period of 50 days following exposure to the virus (47), suggesting a more significant role for mucosal immunity in this species.

**Foot and mouth disease vaccines**

*Production of foot and mouth disease vaccines*

FMD antigens are typically produced in large-scale suspension or monolayer cultures of baby hamster kidney cells (BHK-21) and the filtered product is inactivated with an aziridine, binary ethyleneimine (7). The BHK process has largely replaced the Frenkel method of vaccine production, which was developed in the early 1950s and uses primary bovine tongue epithelial cells in suspension culture (9). This inactivated product is mixed with adjuvant(s) to enhance the immune response. Usually the adjuvants are aluminium hydroxide (Al(OH)$_3$) gel and saponin or a mineral-oil-based formulation which produces an emulsion. The former is for use in cattle, sheep and goats and the latter is used for pigs because of their poor response to Al(OH)$_3$/saponin vaccines. Oil-based vaccines are also used with cattle and, occasionally, with sheep and goats, and are particularly popular in Latin America because of the superior duration of immunity achieved. Inactivated FMD antigens may be concentrated by a number of methods, including polyethylene glycol (PEG) precipitation and ultrafiltration. Highly concentrated antigens are particularly appropriate for use as strategic antigen reserves because of their ease of storage and stability at very low temperatures (36). Clearly, FMD vaccines are relatively crude products and contain high concentrations of cellular protein (approximately 1,000-fold more cell protein than virus; T.R. Doel, unpublished findings), as indeed do concentrates made with PEG 6000 and ultrafiltration. Very pure antigen concentrates may be made by cycles of adsorption and elution with polyethylene oxide (45).

*Application and testing*

The most commonly vaccinated species is cattle, and it is recommended practice to vaccinate calves with aluminium hydroxide/saponin vaccine at four to five months of age, followed by a second vaccination one month later. The initial delay in vaccination allows maternally derived antibody to fall to levels which will not interfere with the response to the vaccine. It has been claimed that mineral-oil-adjuvanted vaccines could be applied as early as two months, when more than 50% of calves with maternally derived antibody demonstrated protective levels of antibodies (6). In the absence of maternally derived antibody, it has been shown that cattle respond well to vaccination as early as one week of age, in terms of both antibody and protection (80). Furthermore, the responses of animals ranging in age from one week to eighteen months were broadly equivalent. There is also some contradictory evidence that calves respond considerably less well to FMD vaccination than adult animals (78). Thus, the same vaccine in approximately six-month-old calves gave 1.7 PD$_{50}$ compared to 10 PD$_{50}$ for three to seven year-old animals.
Thereafter, animals are vaccinated at least annually and often every four to six months to maintain adequate levels of protection. The oil vaccines used for cattle may permit an annual revaccination regimen from two years of age, because of the superior duration of immunity conferred (5). Many vaccines contain more than one valency, for example, mixtures of A24, O1 and C3 viruses are widely used in South America, to cover against the likely strains in the field. In this regard, it should be emphasised that there is no evidence that using more than one serotype in the vaccine causes either enhancement or competitive inhibition of the immune response to any other serotypes in the same vaccine (13).

The two most important tests of FMD vaccines are safety and potency. Safety, specifically freedom from infectious virus, is best assessed in vitro by measuring the kinetics of inactivation and carrying out a final innocuity test on a representative sample after the full inactivation period. In a semi-logarithmic plot of the kinetic data, the whole, or at least the last part, of the inactivation line should be linear, allowing the extrapolation of virus titre to the end of the inactivation period (9). The maximum acceptable infectivity, according to the current European Pharmacopoeia Monograph on FMD, is less than one infectious virus unit per 10,000 litres of bulk antigen (99). The same monograph also describes the in vivo safety test in which each of three cattle are inoculated intradermally with 2 ml of vaccine. The animals are observed for not less than four days, at which time three full doses are administered by the prescribed route. A vaccine fails the test if there is any evidence for replication of virus. Moreover, any undue toxicity or local reaction disqualifies the vaccine. While the cattle test satisfies the requirement for control of the finished product, its value in detecting residual infectivity is questionable. As the most common route of vaccination, namely the subcutaneous route, is generally less sensitive than the tongue route for the detection of virus (between 4 and 250,000 times less sensitive, depending on the virus strain [59]), it can be argued that only 6 ml of a particular batch of vaccine is effectively checked for infectivity of the final product.

The potency of FMD vaccines is usually assessed in cattle, although in vitro assays are becoming more acceptable, provided a correlation with protection has been established (31). Two basic types of potency test are commonly used. The most recent version of the European Pharmacopoeia Monograph indicates the use of six-month-old cattle, or older, arranged as three groups, with at least five cattle per group (99). Instead of preparing a dilution series of vaccine, as was done previously, a different volume of vaccine is administered to each group, according to the route prescribed on the bottle. The dose interval should not be greater than fivefold. Three weeks after vaccination, the vaccinated animals and a control group of two non-vaccinated cattle are challenged intradermally with $2 \times 0.1$ ml of a suspension containing 10,000 ID$_{50}$ of cattle-adapted virus, which is fully virulent and appropriate to the virus type in the vaccine under test. Animals are observed for eight days. Unprotected animals show lesions at sites other than the tongue and control animals must develop lesions on at least three feet. The pass-mark is 3 PD$_{50}$. The test most widely used in South America employs 16 cattle which are vaccinated with the prescribed field dose. Otherwise, the challenge is as above, and a vaccine must protect 75% of the animals to pass the test (Argentina requires 81%). High-quality vaccines are capable of suppressing virus replication at the sites of inoculation as well as the febrile response.

A number of in vitro antibody assays have been developed as alternatives to challenge experiments in cattle (31). These include the enzyme-linked immunosorbent assay (ELISA) and various versions of the tissue culture neutralisation test. However,
a note of caution must be added. Sera are occasionally encountered in which protection would be predicted but is not observed, and vice versa. This is particularly the case with sera from some experiments with novel FMD antigens such as synthetic peptides (30), and has been attributed to the affinities and isotypes of the antibodies induced (76, 96). Moreover, it would be wrong to dismiss this problem as peculiar to novel FMD vaccines, bearing in mind both practical experience and the large number and complexity of variables involved in the immune response (Table I). Probably the best procedure for resolving such problem sera is a passive immunity test in suckling mice (54, 77), which at least allows an immune response to be measured in an environment where phagocytic cells can participate (68).

**Vaccine-induced immunity: general aspects**

FMD antigen harvests used to prepare either Al(OH)$_3$/saponin or mineral-oil-adjuvanted vaccines contain a high proportion of irrelevant proteins and small quantities of structural and non-structural proteins of FMDV. The structural proteins include whole virus particles (146S particles) and various subunits, including natural empty particles (75S) and pentameric clusters (12S) of virus proteins (VP) VP1, VP2 and VP3. The immunogenicity of the 146S particles exceeds that of the 75S particles (found predominantly in the A serotype viruses) and the 12S particles by approximate factors of 10 and 100 respectively (33). The integrity of the 146S particle is thus crucial to the efficacy of a vaccine and poor thermal stability probably accounts for the ineffectiveness of some FMD vaccines (32). Another aspect of antigenic integrity is the VP1 protein within the 146S particle. Many enzymes, including trypsin, cleave important antigenic determinants on VP1 and, while not affecting the stability of the capsid, seriously reduce the immunogenicity of the virus and, hence, the vaccine (34). 146S particles of different serotypes vary considerably in their immunogenicity, for reasons which probably include capsid stability (32) but also other undefined properties of each virus. This is reflected in the quantities of each strain incorporated into a particular vaccine and the potencies achieved, and it is common practice to use four to five-fold more of an O virus than C and A viruses. Furthermore, the resulting potencies of the A and C components of the vaccine are often considerably higher than the potency of the O component (36). Thus, Black et al. reported that A$_{24}$ Cruzeiro antigen was thirty-fold more immunogenic than O$_1$ Campos with C$_3$ intermediate (13). Finally, antibodies induced by O$_1$ vaccines appear to be qualitatively inferior to those induced by C and A viruses (85). That is, more antibody is needed to protect against challenge. Related to this topic, and part of the general mythology of FMD vaccines, is the belief that the Frenkel procedure produces a vaccine superior to the BHK system. Explanations range from the greater stability of the antigen, because of cross-linking of proteins of the virus capsid by formaldehyde inactivation, to trace amounts of live virus due to incomplete inactivation. Unpublished work comparing the different vaccines made in Argentina on the basis of immunoassays of cattle sera indicates a further possibility (J. La Torre, personal communication), that the antibodies induced by Frenkel vaccines may be qualitatively superior to antibodies from BHK-21 vaccine experiments. It is possible that the high proportion of proteins of non-bovine origin in the latter vaccine adversely modulates the immune response. The same author also suggested that qualitative differences may be related to the type of vaccine formulations used.

The consequences of VP1 cleavage or capsid disruption on vaccine efficacy point to the practical importance of conformation-dependent epitopes on the surface of the
virion. Both conformation-dependent and independent epitopes have been and are being mapped on various serotypes with the aid of monoclonal antibodies (MAbs), MAb-escape mutants and, in some cases, crystallographic analysis (65, 67). Thus, in the case of the O serotype, five major sites have been described for the virus, although two of these have sequences in common (26). Because of the importance of virus capsid integrity in conventional vaccines, it may seem surprising to an outsider that so much work has been published on recombinant VP1 and synthetic VP1 peptide vaccines (30, 62), particularly when it is realised that relatively few of these novel vaccines conferred protection and such protection was at levels greatly inferior to those of whole virus preparations. Nevertheless, this approach will undoubtedly continue to stimulate the vaccine development field because of the novel opportunities it offers for antigen expression and precise exploitation of the immune response.

On account of their impure nature, FMD vaccines contain undefined quantities of non-structural proteins coded by viral nucleic acid. While these proteins are not believed to influence the immune response to FMDV significantly, antibody responses against such proteins have been used to assess the likelihood of virus persistence in cattle. Specifically, anti-VIA (virus infection-associated antigen, which contains the RNA polymerase of the virus [79]) antibodies were believed for many years by some workers to be indicative of previous infection. This was refuted by a number of reports in which anti-VIA responses were demonstrated after repeated vaccination with inactivated FMD vaccines (87). It appears that antibody titres against several, rather than one, non-structural proteins, may be a better indication of the likelihood of persistence of virus in cattle (11).

IgM is reported to develop from two to four days post vaccination of cattle (1), and may persist for a considerable time (more than 80 days [17]). The titres also appear to be higher in vaccinated animals than in infected animals, without a distinct peak (1). IgM has been shown to be more cross-reactive than IgG isotypes in neutralisation assays (50). IgG1 was recorded after four days and increased throughout the sampling period of 40 days, whereas IgG2 developed after nine days with a similar profile and tended to peak at 35 days. However, there are two caveats which should be mentioned. The first is that the rate of development and the absolute titres of the different isotypes of antibody are almost certainly dependent on the adjuvant used (Al[OH]3/saponin was used in the study by Abu Elzein and Crowther [1]), and the second is that discrimination between isotypes, particularly between IgG1 and IgG2, is difficult unless highly specific MAb anti-isotypes are used (76). Whether IgG1 and IgG2 isotypes have different protective capacities against FMDV is not known, but it is difficult to believe that there will not be slight differences at least, other characteristics, such as antigenic specificity, titre and avidity, being equal. In this regard, it has been reported that FMDV vaccines induce more IgG1 than IgG2, whereas peptide vaccines generally induce more IgG2 than IgG1, suggesting that IgG1 is a more relevant antibody to protection of cattle, given the superiority of virus vaccines over peptide vaccines (76). It should be noted that the peptide vaccines used in this study induced high levels of neutralising antibody but only low levels of protection. If, indeed, bovine IgG1 is superior to IgG2 in protection against FMD, then the possibility exists that the presence of high concentrations of or high-affinity IgG2 may be counter-productive by binding to the virus and preventing the attachment of IgG1. The complicating factor in attempting to identify the different characteristics of antibodies which contribute towards protection is that the researcher is obliged to work with a polyclonal antibody population, composed of a multiplicity of antibodies varying in
titre, isotype, affinity and specificity. The dissection of this population represents a formidable task. On the question of affinity, the most useful data relate to peptide vaccines, where it has been shown that high-affinity anti-peptide antibodies were more likely to be protective than low-affinity antibodies (96).

Protective levels of antibody produced by a single vaccination tend to be short-lived, lasting only a few months, although this depends very much on the type of vaccine and the possible interference from maternally acquired antibodies. Duration of antibody after the first or subsequent revaccinations varies considerably from one vaccine preparation to another (see following section). While optimum levels of protection are usually recorded from 21 to 28 days after vaccination, several reports indicate protection of cattle and pigs within a few days of vaccination (40, 42, 57). The early protective mechanism is not known but does not appear to be antibody because of the very low or background titres recorded soon after vaccination.

Mucosal immune responses of cattle due to vaccination are very different from those seen with infection. After the inoculation of oil emulsion vaccine, ELISA of pharyngeal fluid showed a rapid development of IgG but a more delayed IgM response (48). Subsequent vaccinations at approximately 60-day intervals produced a series of IgM and IgG peaks but, in contrast to parallel experiments with infected animals, IgA was not apparent at any time. However, Garland observed that neutralisation was associated with IgA as well as IgG in the secretions of a steer after a third dose of Al(OH)₃/saponin vaccine, approximately one year after first vaccination (50). This suggests that the type of vaccine and/or time interval between vaccinations may influence whether or not secretory IgA is induced. In the experiments of Francis et al. (48), neutralising activity in the pharyngeal fluid did not appear to coincide with the early appearance of IgG but rather peaked at the second vaccination time point (63 days), whereas Garland reported that neutralisation was principally associated with the IgG₁ class in once or twice-vaccinated animals (50). It is interesting to note that the class of antibody which transferred most effectively from the blood of passively immunised cattle to the mucosae of the oropharynx was IgG₁, although the author pointed out that the original source of immune serum was predominantly IgG₁ and IgG₂ (50). There was also evidence that this selective transfer may have been assisted by increased vascular permeability due to hypersensitivity reactions. The mucosal immune response following vaccination with Al(OH)₃/saponin preparations does not appear to be significantly different from that seen with oil vaccines, although the antibody titres were lower (48). Francis et al. also noted Garland's earlier work and suggested that the neutralising activity in the pharyngeal fluid (and hence the titres of specific IgG and IgM) was due to serum transudation (48, 50). In another experiment, in which cattle were vaccinated shortly before challenge, the ELISA antibody/neutralising antibody responses most closely resembled those of animals which were infected without prior vaccination, i.e. a significant but later development of IgA only (48). From this work, it would be concluded that one or two rounds of conventional FMD vaccine do not normally induce significant levels of IgA in the oropharynx, whereas serum-derived neutralising IgG₁ may reach the mucosae. Additional rounds of vaccination with a conventional product or, perhaps, one application of a highly potent vaccine may result in the appearance of secretory IgA. In this regard, IgA was demonstrated in the nasal fluid of four sheep, nine days after vaccination with six times the normal dose of aqueous vaccine (51).

Neutralising antibody responses were observed in pig nasal fluid within 3 to 7 days of injection of an oil vaccine (water-in-oil or water-in-oil-in-water), but the titres
thereafter were relatively unaffected by subsequent vaccinations at 56 and 117 days post vaccination (dpv) (47). In contrast, the virus-neutralising antibody titres increased after each vaccination; the water-in-oil preparation being the most effective. Unfortunately, the authors, Francis and Black, were unable to identify the antibody classes responsible for the neutralising activity in the pharyngeal fluid. An important conclusion from this paper was that the magnitude of the mucosal immune response in the pig following vaccination may explain why correlations between protection and serum antibody levels are less reliable in this species than those established with cattle (47).

Vaccination with Al(OH)₃/saponin vaccines induces a population of T-lymphocytes in bovine peripheral blood which proliferates in vitro in response to optimum concentrations of FMDV (1.0 µg/ml) (17, 19). Such proliferative responses tend to be relatively weak after only one vaccination (49, 100), and it is necessary to vaccinate at least several times before high stimulation indices are obtained. Surprisingly, perhaps, peripheral blood T-cell responses following infection do not appear to be superior to responses achieved with vaccination (49). The duration of FMDV-specific T-cell immunity contrasts with that generally observed for neutralising antibody, and was reported as being 20 months for one particular twice-vaccinated animal (19). Furthermore, the possibility exists that non-circulating FMDV-specific T-cells are resident for longer periods. One of the most significant features of the bovine T-cell response to FMDV is the cross-reactivity among the different serotypes (17, 19, 49, 100), in clear contrast to the serum antibody responses. For example, peripheral blood lymphocytes from an animal previously vaccinated with O serotype virus proliferate strongly and often equivalently when stimulated in vitro with A serotype virus (19). This cross-reactivity was attributed to conserved epitopes within VP1, VP2 and VP3 of FMDV. Finally, there does not appear to be any correlation between neutralising antibody titres and T-cell proliferative responses (100).

Earlier studies with virus alone prompted investigations to locate and define T-cell epitopes within the structural proteins of FMDV with particular reference to synthetic FMD vaccines. Collen et al. screened a number of VP1 synthetic peptides and identified an immunodominant T-cell epitope between residues 21 to 40, which was recognised by 7 of 19 virus-vaccinated cattle (20). Sequences containing the 141-160 region of VP1 were less effective in T-cell proliferation assays. In fact, studies from the Pirbright Laboratory were generally unsuccessful in generating strong T-cell responses to peptides containing the VP1 141-158 sequence or in showing their recognition by T-cells from virus-vaccinated cattle (17, 20, 49; and T.R. Doel, unpublished findings). For reasons which are not clear, Van Lierop et al. have had greater success with peptide-immunised cattle, although a multiply virus-vaccinated animal gave little or no T-cell proliferation to peptides (100). This work demonstrated that the 141-156 and 200-213 sequences of VP1 induced bovine T-cells, and that the stimulation indices were particularly high when the immunising peptide was in the form Cys-Cys-(200-213)-Pro-Pro-Ser-(141-156)-Pro-Cys-Gly. In addition, it was clear that T-cells from peptide-vaccinated cattle recognised homologous virus in the case of the 141-156 sequence, and heterologous as well as homologous virus in the case of the 200-213 sequence. Therefore, both peptides contain T-cell as well as B-cell epitopes (see 'Serotype specificity'). Finally, MHC restriction of bovine T-cell responses to synthetic FMD peptides has been demonstrated (52), indicating the possibility that simple FMD antigen vaccines may fail to stimulate the immune response of some animals. If this is the case, it will be necessary to define and use a
T-cell epitope which is recognised by a broad spectrum of cattle of different genetic backgrounds or, alternatively, to devise synthetic antigens which contain several or more T-cell epitopes which are recognised as a group by the majority of the target population.

T-cell responses against whole virus and isolated proteins have also been demonstrated in C serotype-vaccinated inbred miniature pigs (91), including the observation that the response was heterotypic. Following two vaccinations, the T-cell responses persisted for at least one year, although the quantities of antigen required for in vitro proliferation were particularly high (40-200 µg/ml) in comparison with the cattle experiments referred to above.

Duration of immunity

Much of the information on the duration of immunity has been obtained in the most vaccinated species, cattle. However, the more limited data from pigs and sheep do not differ greatly with respect to the rate of antibody development and decay. Not surprisingly, there is considerable variation in the reported duration of immunity following FMD vaccination because of variables such as vaccine quality and adjuvants used. This is reflected in the different vaccination regimens used by different countries. For example, revaccination may be done every four, six or twelve months to maintain antibody levels. It is generally believed that the frequency of immunisation appropriate for Al(OH)₃/saponin vaccines can be reduced in the case of oil-adjuvanted vaccines because of the more prolonged antibody response. This is suggested by the work of Gomes et al. (55). In this experiment, 30 cattle which had been vaccinated three times, at six-month intervals, with oil-adjuvanted vaccines, were challenged 13 months after the last vaccination. A high level of protection was predicted according to the pre-challenge antibody levels and, indeed, 29 animals were fully protected. Nevertheless, it would be misleading to carry this general assumption too far because of the very different oil formulations available internationally and the proven high quality of some Al(OH)₃/saponin products.

As the duration of effective immunity will depend not only on the establishment of memory following vaccination, but also on qualitative aspects of the induced antibody and, particularly, the initial magnitude of the immune response, it is worthwhile briefly reviewing some of the classical work with an experimental antigen (94). Using 2,4-dinitrophenyl-bovine γ-globulin in guinea-pigs, it was shown that large doses of antigen gave an initially high concentration of serum antibody which peaked early in the immune response (at 13 days), decreasing subsequently to relatively low levels of low-affinity antibody. In contrast, smaller doses of antigen gave initially low levels of antibody which continued to rise until much later in the immune response, when the levels, but particularly the affinities, greatly exceeded those obtained with high concentrations of antigen. With very low doses of antigen, the amount of antibody synthesised and its affinity decreased throughout the course of the experiment. It would appear, therefore, that an optimum dose of this antigen exists with respect to development and persistence of high concentrations of high-affinity antibody. Furthermore, boosting the immune response produced the highest affinities when the primary response was elicited by an optimum dose and was favoured by an increased time interval between the two immunisations. The study of Siskind and Benacerraf provided two important conclusions, as follows (94):

- the suggestion that supra-optimal concentrations of antigen may drive cells into terminal differentiation to antibody-forming cells, rather than general cellular
proliferation (including the pathways to memory cell development?)

- the striking coincidence that the optimal dose of antigen with respect to antibody concentration also produced the highest-affinity antibody.

Some of these experimental observations can also be related to FMD vaccines. In general, FMD vaccines give peak antibody responses in cattle 21 to 28 days after vaccination but more rapid development of peak antibody titres around 14 dpv is occasionally observed (40, 89). Antibody titres may decline rapidly or remain relatively high for several months before falling below protective levels. The latter type of antibody profile has been observed more frequently with some oil vaccines (71), but work by Fish et al. demonstrated that Frenkel Al(OH)₃ vaccines were capable of producing prolonged antibody responses (46). This work showed that cattle selected from the annual revaccination programme of the Netherlands during the 1960s initially produced peaks of neutralising antibody lasting approximately twelve weeks following revaccination, after which antibody titres levelled off and remained at a plateau for up to 44 months post primary vaccination. Interestingly, the duration of antibody response was not significantly affected by the number of revaccinations (2, 3, 4, 5, 6, 7 or greater), whereas the plateau heights were increased slightly each time (approximately 0.1 log₁₀ per revaccination cycle up to 4/5 revaccinations). In the case of sheep, it has been shown that one type of oil-adjuvanted vaccine gave improved duration of antibody titre compared with Al(OH)₃/saponin vaccines, but this was not reflected in the protection against challenge at 9 months post vaccination (13 sheep protected/14 sheep challenged and 13 sheep protected/13 sheep challenged, respectively) (72).

Clearly, the rate of antibody decay following peak titres can have a dramatic influence on the 'window' of susceptibility of an animal. The gradual development and decay of antibody titres seen with some FMD vaccines resembles that reported with optimal levels of 2,4-dinitrophenyl-bovine γ-globulin, whereas relatively brief peak responses are more reminiscent of the use of supra-optimal levels of antigen. From experiments in cattle with Al(OH)₃/saponin FMD vaccines containing between 7 and 329,760 ng of 146S per dose, it was concluded that the primary response was dose dependent but that there was no evidence of low-dose tolerance or high-dose immunological paralysis (89). While the highest primary antigen doses produced the highest antibody titres, the secondary responses were generally superior following primary doses of 42 or 254 ng virus. An important consideration which, to the knowledge of the author, has not been examined is the possible interference/modulation of the immune response to the virus by the high concentration of cellular proteins present in conventional FMD vaccines.

One of the most striking variations in the immune response to FMD vaccines is the extent to which the immune response is boosted by subsequent vaccinations. The increase in neutralising antibody titre may often exceed 1.5 log₁₀ (84), whereas little or no boosting of the immune response is apparent in data from other reports (48, 88). Indeed, some antibody profiles of cattle which are vaccinated every six months look more like a succession of primary responses rather than a continuously maturing immune response (T.R. Doel, unpublished findings). This suggests that the initial and subsequent vaccinations did not stimulate the development of immunological memory. Given this type of immune response, it is clear that animals would not be protected for more than approximately three months in any one vaccination period. It has been demonstrated that antibody titres following revaccination are also dose dependent and that the difference between primary and anamnestic responses was most marked when
low-antigen doses were used initially (12). The suggested explanation was that high initial antibody titres limited anamnestic responses, either by sequestering antigen or by regulating the immune response more directly. An interesting comment from this work was that priming of the immune system was saturated by less than 254 ng virus. There is also one final cautionary note to be made in terms of conclusions drawn from antibody titres. As Cowan observed (24), the kinetics of antibody formation using neutralisation assays may be misleading because what is measured is a combination of both the rate of antibody formation and the development of avidity.

It is worth commenting that the official acceptance tests for FMD vaccines relate to the ability to protect cattle 21 to 28 days after one dose, rather than to suitability for regular use in mass vaccination programmes, the most common application of the product. Indeed, given sufficient experimental work, it is likely that two different products could be developed. The objective of the first vaccination would be to prime the immune system for optimum performance following revaccination and not necessarily to develop a rapid and protective immune response. Equally, booster vaccine would not be assessed on protection after one dose, but rather on the ability to boost the immune response.

While it is undeniable that FMD vaccines do not give long-lived immunity, and that it is necessary to revaccinate regularly to maintain protective levels of antibody, there are several misconceptions in this area. In fact, many vaccines are probably much better than is commonly thought and perceived deficiencies are based partly on comparisons with other inactivated vaccines, such as poliovirus (IPV), and reports of very long-lived antibody titres following recovery from FMD (28, 50). In the case of IPV, which confers a long duration of immunity, the comparison is not legitimate. The simple objective of IPV vaccination is to prevent the development of poliomyelitis. Minor gastroenteritis, because of inadequate gut immunity, is of little consequence to a thoroughly vaccinated population, provided systemic immunity is satisfactory. Furthermore, poliovirus infection gives sufficient time for the systemic immune response to be boosted. In contrast, significant replication of FMD in the oropharynx is not desirable, considering the possibility of rapid dissemination of the disease to non-vaccinated contact animals (pigs, sheep, goats and partially immune cattle). Equally, the rate of development of clinical FMD in an experimentally challenged host is so rapid — vesicles on the feet may develop 36 hours after contact challenge (T.R. Doel, unpublished findings) — that there is little opportunity for the immune response to be boosted to protective levels. In respect to immunity following infection, it is important to consider the possible role of persistence with convalescent cattle, in which trace amounts of virus probably facilitate the full maturation of the T- and B-cell responses, including memory. This hypothesis is strongly supported by the observation that pigs, which do not become persistently infected, only remain protected for about six months following infection (53, 70). Nevertheless, the question remains whether FMD vaccines are, generally, as effective as they might be, taking into account observations such as low or non-existent boosting of antibody titres.

Because it would be highly desirable if one dose of FMD vaccine protected an animal for life, it is worth considering some of the ways in which this could, theoretically, be achieved. All the following approaches rely on one central concept: the continuous or pulsed stimulation of the immune system with the antigen of interest. In this regard, constant antigenic stimulation has recently gained greater acceptability among immunologists as one of the possible mechanisms responsible for maintenance of immunological memory (69).
a) Sustained delivery of FMD antigen from an inert, i.e. non-replicating, carrier. Polymeric materials are available which allow the gradual or even pulsed delivery of antigens but these have not been evaluated with FMDV, to the knowledge of the author (81). One of the potential problems with FMDV is the relatively poor stability of the virus, which could hinder preparation of the sustained-release material and would probably reduce the efficacy in vivo because of virus degradation. If stability was not a problem, it is probable that very potent vaccines could be achieved. Studies by the author with simulated slow release of virus, in which cattle were inoculated every other day for 20 days, indicated a higher and more prolonged immune response than the same payload of virus in Freund's complete adjuvant (T.R. Doel and R.F. Staple, unpublished findings). However, a polymeric delivery system would be much better suited to a stable FMD antigen, such as a synthetic peptide or recombinant protein, although preliminary experiments with the former, delivered by implanted osmotic pumps, did not demonstrate significant antibody responses in guinea-pigs (T.R. Doel and R. DiMarchi, unpublished findings). Finally, a simple but important factor, which is often overlooked in the development of even conventional FMD vaccines, as well as in more sophisticated antigen delivery systems, is cost. While it may seem logical to pay two or three times as much for a vaccine which confers more durable immunity, it must be remembered that the market for the vast bulk of FMD vaccine is in developing countries, where public or private resources are often insufficient to support comprehensive vaccine campaigns with existing products.

b) Delivery of FMD antigen in a replicating vector such as infectious bovine rhinotracheitis virus (IBRV), with particular respect to establishing immunity in the upper respiratory tract. Kit et al. inserted a DNA sequence, comprising bovine growth hormone upstream of the O1 Kaufbeuren VP1 sequence 200-213 linked through Pro-Pro-Ser to VP1 141-158, at the N-terminal end of the non-essential IBRV surface glycoprotein gIII gene (61). The FMDV peptide-gIII fusion product was found to be expressed on the surfaces of virus and virus-infected cells. In a pilot experiment with the recombinant vaccine, protection against intradermolingual challenge was claimed at 21 days post vaccination, although the data were not presented. Unfortunately, this vector did not appear to be used in any further experimental work and, more specifically, no attempt was made to examine the immune response of cattle following administration of the vector virus via the upper respiratory tract.

c) Use of vaccines made with 'attenuated' strains of FMDV. Given the ability to manipulate DNA complementary to the RNA of FMDV and recover precisely engineered infectious virus (103), novel attenuated vaccines should be feasible and may give high-quality immunity. However, attenuated FMD vaccines would have a number of potential disadvantages. Firstly, export markets would not accept meat or animals if a persistent FMD infection had been detected, regardless of the pathogenicity of the virus. It is assumed that an attenuated FMD vaccine which did not cause some degree of persistence would not protect for any longer than a good inactivated vaccine, and would therefore be pointless. Furthermore, it could very well be necessary to eradicate the vaccine strain before an FMD-free status was acknowledged internationally. Secondly, there is the finite risk of recombination occurring in the field and the consequent ‘rescue’ of a virulent strain of virus. Intertypic recombination has been proven in the laboratory (66), and Krebs and Marquardt published data to suggest that the field isolate O1 Burgwedel/1987 was a recombinant between O1 Kaufbeuren and a C1 strain virus (64). A different type of rescue mechanism is suggested by the work of Mason et al. (74), who produced mutant
viruses which contained changes within the Arg-Gly-Asp sequence of VP1 and were unable to attach to BHK cells. Antibody-mediated complexing of these viruses allowed them to infect Chinese hamster ovary cells expressing the receptor for Fc (crystallisable fragment) of Ig. Nevertheless, one of the saving graces of the recombinant DNA approach is that it should be easier to design viruses systematically which do not suffer the problems of reversion or species-specific virulence observed with conventional attenuated FMD vaccines.

In conclusion, there appears to be evidence of the failure of some FMD vaccines to establish what would be considered a typical immune response – namely, the very significant increase in antibody titres which usually follows a second or subsequent vaccination. A much higher antibody titre after boosting would, if nothing else, provide protective levels of antibody for slightly longer before the antibody fell below threshold levels for protection. Whether these observations reflect the unavoidable reality of immunisation with an inactivated antigen or specific deficiencies with some FMD vaccines will require further immunological studies. It could be that duration of immunity could be extended by at least a few months if more was known about the conditions which favour priming of the immune response to FMD. With respect to alternative technologies, a live virus or bacterial vector carrying FMD antigens appears to offer the most promising approach, although the problem remains that the quality of immunity so far induced by simple antigens, such as the FMDV VP1 peptide Cys-Cys-(200-213)-Pro-Pro-Ser-(141-158)-Pro-Cys-Gly, is significantly inferior to that induced by whole virus.

Persistence

Conventional FMD vaccines do not prevent the development of the carrier state. Indeed, one of the objections to maintaining a vaccine campaign in a country, after apparent eradication of FMD, is the implications for trade because of the possibility of disease persisting in animals under the cover of vaccine. However, there is evidence to suggest that single doses of high-quality vaccines or regular vaccination can reduce the incidence significantly (3, 40, 50), and it seems very likely that a combination of the two approaches would greatly aid in solving the problem of persistence. An alternative method would be to develop novel vaccines, and several such vaccines may be envisaged, taking into account the limited knowledge of the phenomenon.

Data on the immune responses of carrier and non-carrier cattle indicate generally that carrier animals maintain higher titres of neutralising antibody than convalescent non-carriers (92), with respect to both the levels in serum and in oesophageal-pharyngeal fluid (73). Clearly, therefore, FMDV is able to infect, replicate and establish the persistent state in privileged sites in the oropharynx before the immune system has an opportunity to mount an effective response. Once established, the virus is able, presumably, to provide a more or less constant stimulus to the immune response until FMDV eventually dies out and the antibody titres fall correspondingly. One flaw in the hypothesis of constant antigenic stimulation is that a small but significant percentage of seronegative, persistently infected animals have been reported (58, 97). One possible explanation for the absence of specific serum antibodies is that these animals are in a state of low-dose or high-dose tolerance, although there is no experimental evidence to support this hypothesis. A second explanation is suggested by the findings of Sutmoller et al. (98), who reported that a high percentage of cattle are infected with bovine enterovirus (BEV) and that FMDV-BEV hybrids, in which the FMD RNA is encapsidated by BEV structural
proteins, are produced in the oropharynx following infection by FMD. Thus, these FMDV-BEV hybrids would not be expected to replicate readily in the oropharynx or elsewhere because of the high titres of antibody against BEV, but would allow the isolation of infectious FMDV in tissue culture of oesophageal-pharyngeal samples. Indeed, the authors suggested that BEV significantly modulated the development of clinical FMD in some animals. The contribution of such FMDV-BEV hybrids to the maintenance of the persistent state is not known but the problem is clearly complex and prevention of persistence presents a daunting task for any vaccine.

The most obvious strategy would be to develop a vaccine which maintains high levels of neutralising antibody at the main portal of entry of the virus, the oropharynx, so that the infection has no opportunity to replicate locally. It is difficult to believe that a single dose of a parenterally administered non-replicating vaccine (whether it was conventional virus or a synthetic or expressed antigen) could achieve this, unless it were very potent. Although reinoculation of the same serotype in a live or attenuated form also appears to cure the persistent state in many animals, the use of an 'attenuated' virus vaccine would not be an advisable strategy for the reasons given in 'Duration of immunity' (4, 73). The better option would be a non-pathogenic bacterium or virus which was engineered to express specific FMDV antigens and which replicated at low levels in the oropharynx for a considerable period of time. Furthermore, the choice of a bacterial vector would at least permit the 'eradication' of that vector by antibiotic treatment if it became necessary.

The report that infection or vaccination with a second serotype appears to reduce existing as well as potential persistence suggests that replicating recombinant vaccines specifically expressing non-structural proteins, such as the RNA polymerase of FMDV, into the oropharynx could prevent the establishment of persistence (40). The rationale for identifying non-structural proteins is based on the assumption that an antibody response is involved in the intertypic cure or prevention of persistence (40). In this case, the antigenically conserved non-structural proteins are more probable candidates than the antigenically variable structural proteins in boosting responses between serotypes. Of course, another explanation for these observations is the induction by the second virus of an alternative defence mechanism, such as interferon. If the previously mentioned FMDV (RNA)-BEV (protein) hybrids play a role in the maintenance of the persistent state, then the stimulation of antibodies against the non-structural proteins of FMDV could also be more effective in limiting or preventing replication of FMD RNA in this form (98).

Finally, there is the question of the importance of persistence and the need to avoid or prevent it. Despite the undesirability of the phenomenon, it is possibly overstated as a problem. Certainly, good-quality conventional vaccine campaigns would be expected to reduce the level of persistence to a minimum. Moreover, it has proven extremely difficult to demonstrate transmission from one animal to another under highly controlled conditions, and there is only circumstantial evidence from the field to indicate that persistently infected animals have a role in the maintenance and dissemination of the disease (92). It is arguable, therefore, that persistence is a political problem rather than an actual one, in situations where cattle have been regularly vaccinated with high-quality products containing the appropriate strains of FMDV.

Serotype specificity

FMDV shows a high degree of antigenic variability, as evidenced by the seven serotypes and numerous subtypes distinguished to date. However, the distinction
between subtypes is less clear than between serotypes, and is essentially quantitative rather than qualitative (86). Furthermore, the antigenic relationships within a serotype may be quite distant (e.g. A\textsubscript{24} and A\textsubscript{22}) or relatively close (e.g. C\textsubscript{1} and C\textsubscript{3}). This has important implications for the choice of a suitable vaccine strain and it is essential that the selection is made on the basis of the relationship with field strains using accepted serological methods rather than historical or other precedents.

In contrast to the situation following multiple infection of cattle (23), the serotype barrier is considered to be inflexible as far as vaccines are concerned, although the author is not aware of a comprehensive study which allows this conclusion. Thus, it is commonly stated that animals vaccinated with one serotype of FMD are not protected against the remaining six serotypes. One general observation and one specific study support this statement. In the first place, it is clear that the widespread practice of multiple rounds of vaccination with bivalent, trivalent and sometimes tetravalent vaccines does not prevent outbreaks due to antigenic variants of FMD. The specific study reported that each virus serotype within a South American trivalent vaccine acted independently in relation to the immune response, and neither enhanced nor depressed the responses to the other serotypes (13). The serotype barrier for vaccine extends partially to the subtypes. For example, the A\textsubscript{24} and A\textsubscript{22} subtypes are sufficiently distinct antigenically to warrant vaccines developed against each strain. In fact, it is common practice for the strains used in production to reflect the local field situation closely, to minimise the influence of differences between the field and vaccine strains. While it is perfectly realistic to update the vaccine strains to match recent field isolates, it is necessary to appreciate that this has cost implications, because of such factors as the effort and time required, including regulatory issues relating to the registration of new vaccines. Furthermore, it may be difficult to generate a satisfactory vaccine strain from a field isolate because of poor growth in BHK suspension cells, etc., and it may be more judicious to use a less closely related but potent vaccine strain of proven efficacy. In any case, it has been demonstrated that multiple rounds of vaccination with one strain of FMD result in an increased likelihood of protecting against related subtypes (84). However, it must be emphasised that the basic relationship among strains of the same serotype does not change following multiple vaccinations. Simply, the increased titres caused by the boosting of the immune response reach levels which also protect against subtypes related to the vaccine strain (44). The implications of this are that, as antibody titres rise and fall from vaccination to vaccination, protection against a field strain which is closely matched to the vaccine strain will last longer than protection against a related subtype. Finally, it has been reported that attenuated FMD vaccines are capable of conferring broader protection than inactivated preparations (60), although there are a number of disadvantages associated with their use, as this review indicates.

The so-called 12S antigen of FMDV has been implicated in stimulating inter-serotypic antibody responses (16), but it is difficult to reconcile the data with the fact that all but the purest conventional FMD vaccines probably contain a significant quantity of 12S (approximately 50% of the complement-fixing [CF] activity of O\textsubscript{1} BFS 1860 harvests; T.R. Doel, unpublished findings) but do not induce cross-protection (13). A partial explanation for the data of Cartwright et al. (16) is that cross-reactivity between serotypes is not infrequent in the virus neutralisation test (22). In contrast, synthetic FMD peptide vaccines based on VP1, and having the sequence Cys-Cys-(200-213)-Pro-Pro-Ser-(141-158)-Pro-Cys-Gly, clearly induce cross-protection after one immunisation of guinea-pigs and, possibly, two immunisations of cattle (37, 38).
Cross-protection was attributed to the 200-213 sequence at the C-terminus of the protein, whereas the 141-158 sequence was serotype specific. Having said this, it is interesting to note that a large number of monoclonal antibodies raised originally against A_{24} Cruzeiro virus recognise the 141-158 sequences of both A_{24} and O_{1} viruses (39). Thus, studies with monoclonal antibodies and synthetic peptides demonstrate that it is possible to stimulate the immune response with simple antigens to produce cross-reactive antibodies, whereas a complex antigen, such as the whole virus, fails to do this. Whether this failure has any immunological significance or merely represents the inability of the cross-reactive antigens within whole virus to compete and/or survive, and be presented to the appropriate clone of B-cells, is not known. In the former situation, the possibility exists that the cross-reactive epitopes are 'sequestered' by the immune system for recognition as T-cell epitopes, although this seems unlikely from the T-cell studies published. Peptides may have a considerable advantage over large protein complexes because of their antigenic simplicity, allowing the immune response to 'concentrate' on relatively few antigenic sites. However, some workers have expressed misgivings about the value of synthetic peptide vaccines because of this simplicity, suggesting that minor mutations in the challenge virus population would allow escape from the immune system. In fact, in addition to the cross-protection observed with these synthetic peptides, one report indicated that it was considerably more difficult to generate an escape mutant with peptide antibody than with anti-viral antibody (82). Finally, it is noteworthy that the linear order of T- and B-cell epitopes within complex peptides can dictate whether or not a given sequence is recognised as a T- or a B-cell epitope (25, 83). Thus, intelligent manipulation of the immune response with the goal of improved FMD vaccines will rely heavily on a thorough understanding of the mechanisms involved in antigen processing and presentation.

The observation by Cottral and Gailiunas that multiple rounds of infection with different serotypes of FMD can reduce or prevent clinical disease due to other serotypes suggests that vaccines could be developed which confer cross-protection (23). Given the failure of parenterally administered vaccines, it is suggested that oral administration of a 'replicating' antigen would be more appropriate (see 'Persistence'). A further refinement to this approach would be the use of vectors expressing non-structural proteins of the virus which, because of their high sequence conservation among the seven serotypes, might be more effective in preventing broad-spectrum virus replication in the oropharynx.

In conclusion, one of the most promising approaches to stimulating cross-protective antibodies appears to be through the use of synthetic peptides or similar antigens. An interesting strategy to consider would be the application of conventional vaccines following immunisation with a synthetic peptide or recombinant protein so that, hopefully, the cross-reactive antibodies would be induced and boosted while generating antibodies against the other sites involved in neutralisation of the virus.

Stability

Foot and mouth disease virus is a relatively unstable particle, both with respect to pH and temperature (32, 102), and this has clear implications for the shelf-life of vaccines, particularly where cold-chains are not effective. The stability of the virus to pH varies with the serotype. Thus, the strain C Noville is more acid labile than A and O serotype viruses; particle integrity and infectivity being adversely affected below pH 7.0 and 7.5 respectively (T.R. Doel, unpublished findings; 50). In the case of the
A and O serotypes, particle integrity and infectivity are adversely affected below pH 6.5 and 7 respectively (T.R. Doel, unpublished findings; 50). Infectivity is also reduced above pH 8.0 for the A and O serotypes and above pH 8.5 for the C serotype (50). The thermal stabilities of different serotypes of the virus may be ranked, the A and Asia 1 serotypes being the most stable and the Southern African Territories (SAT) serotypes being the least stable. O and C serotypes have intermediate stability (32). Inactivation with acetyleneimine and, probably, binary ethyleneimine (BEI), reduces the thermal stability of all serotypes (32, 56), particularly with the strain O Hong Kong (95). Fixation of aziridine-inactivated FMDV with formaldehyde or glutaraldehyde enhances significantly the thermal stabilities of the capsid (32), allowing the preparation of an effective vaccine with the unstable SAT2 strain, Kenya 227/66 (75). Contradictory results have been reported (90), in which formaldehyde treatment reduced the concentrations of 146S particles in five of six South American vaccine strains and the stability of the same preparations during storage at 4°C, whereas BEI treatment had little effect. It is difficult to rationalise these results with results from other laboratories, but one variable could be the complex mixtures of polymeric aldehyde compounds often present in formaldehyde and glutaraldehyde solutions.

Natural empty particles (75S) of the virus appear to be less thermostable than 146S particles, consistent with their lower immunogenicity (32, 33). In general, thermal stability seems to correlate well with the immunogenicity characteristics of a given serotype (32). However, a complicating factor is that little is known of the influence of adjuvants on the stability of 146S particles, although adsorption to Al(OH)₃ may destabilise some strains (35).

While it is difficult to assess the importance of virus particle stability in relation to the other factors which define the success or failure of an FMD vaccine, there is little doubt that instability represents a potential problem and it is worth considering some of the possible approaches to enhance vaccine stability.

Aldehydes such as glutaraldehyde and formaldehyde are perhaps the simplest way to stabilise virus particles. However, the chemistry of these solutions is complex and variable and it would seem more logical to consider the large family of modern protein cross-linking reagents. Such reagents, while costly, permit cross-linking to be made between specific sites at precise interatomic distances. Of particular relevance is the ever-increasing data base being generated by crystallographic studies on FMDV (2, 65), which should facilitate such an approach.

An alternative approach would be to generate hybrid viruses in which the most important antigens of the unstable strain, such as the G-H loop of VP1, are inserted into the protein framework of a stable FMDV strain, such as A₂₄ Cruzeiro. This approach will be greatly facilitated by the work of Zibert et al. who produced complementary DNA (cDNA) copies of the viral RNA from which infectious virus could be recovered (103).

The various recombinant systems and synthetic peptides referred to earlier also offer the possibility of more stable FMD vaccines. At the same time, it is important to realise that recombinant and synthetic antigens are not always as stable as commonly claimed. In the first place, they may have poor stability in vitro through, in the case of synthetic peptides, oxidation of side chains and other side chain reactions. More importantly, perhaps, they may be more prone to degradation in vivo by cellular enzymes, both at the site of immunisation and during processing by presenting cells of the immune system. It is worth reflecting that native proteins, such as virus
particles, are usually more resistant to proteolysis than denatured/aggregated molecules, such as isolated VP1.

Residual infectivity

Residual infectivity is widely claimed to be a serious drawback to the use of FMD vaccines, although the criticism has been levelled almost exclusively at the older formaldehyde-inactivated preparations. The main reason given for the occasionally reported failure of such vaccines was that the kinetics of inactivation were not first order and the process left a residue of infectious virus. Claims to the contrary (8) were refuted by Bahnemann (7), who cited evidence that formaldehyde-treated virus had a markedly extended incubation period for the first cycle of replication in cell culture. Thus, tissue culture tests to measure innocuity of formaldehyde-treated virus could not be considered valid if these tests did not cover this extended incubation period (approximately 12 days). The discovery and development of the aziridines as first-order inactivants for FMD circumvented the residual infectivity problem. Initially, acetyleneimine was widely used, following the pioneering work of Brown and Crick (15), but this was replaced by ethyleneimine (EI) and BEI (7). The different terminology is used to distinguish between the pure form of the chemical (EI) and the product made from bromoethylamine hydrobromide and sodium hydroxide immediately prior to use in the production of FMD vaccine (BEI). While aziridines are inherently more effective inactivants than formaldehyde, it is essential that the proper procedures for inactivation are used. These procedures include the following:

a) the correct formulation of the inactivant which, in the case of BEI, is made outside the virus vessel in a sealed reaction vessel immediately prior to addition to the virus

b) the addition of the inactivant to the virus, with thorough mixing and later transfer to a second, sterile vessel

c) precise temperature control and effective mixing throughout the inactivation period

d) the taking of regular samples throughout and at the end of the inactivation process and the assay of such samples on sensitive cells (9).

From extrapolation of the inactivation curve, it is possible to determine the maximum number of infectious particles remaining per unit volume after inactivation has finished, as well as a statistically meaningful assay on the final product. The inactivation procedure is not satisfactory unless there is less than one infectious virus unit per 10,000 litres of liquid preparation (99).

The issue of residual infectivity has been used by some workers to justify research and development into safer, more effective FMD vaccines. The obvious candidates are synthetic peptide vaccines and recombinant vaccines based on either bacteria or other viruses. However, it will be clear from this review that novel vaccines do not offer sufficient potency at present to be seriously considered as replacements for conventional FMD vaccines. Perhaps more importantly, there is no reason to believe that a vaccine production facility operating under conditions of Good Manufacturing Practice, and correctly employing the proper procedures for inactivation with BEI/EI and innocuity testing, will ever release a single batch of vaccine which represents a risk to vaccinated livestock.
Concluding comments

There is clearly a wide range of possible approaches to the development of more effective FMD vaccines. However, scientific considerations will be outweighed by the commercial viability of a new product. This unfortunate truth reflects the relatively low profit margins on one of the most important animal vaccines. It is often overlooked that the bulk of the FMD vaccine market is in developing countries, where private and public funds for vaccine purchase are limited and where other problems, including issues of public health, have political precedence. The difficulties of developing a new product are compounded by the increasingly restrictive legislation surrounding veterinary drug registration, which can add a high additional cost before a product can be licensed. This situation is considered to be stifling the development of all but the most potentially profitable products in animal health (101).

One thing is very clear. FMD remains the most important disease of livestock and one of the most difficult to control, nationally and internationally, because of the rapidity and ease with which it can spread. Thus, a serious outbreak in any one country should never be dismissed lightly. It represents not only a threat to neighbouring countries but also a dangerous source of the disease, which could be inadvertently transferred to a more distant trading partner. It is, therefore, in the interests of all concerned to make every effort to ensure that good vaccines continue to be produced, and that scientists and commercial producers are given sufficient incentive to develop improved vaccines which, one day, will enable the world-wide eradication of the disease.

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Résumé : L'auteur analyse la réponse immune du bétail domestique au virus de la fièvre aphteuse, que ce soit après une infection naturelle ou une vaccination, ainsi que les caractéristiques et propriétés des virus et des vaccins de la fièvre aphteuse en termes de protection contre la maladie. L'auteur considère notamment les différents moyens susceptibles d'améliorer les vaccins classiques, ou d'en produire de nouveaux, contre une maladie d'importance majeure.


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Resumen: El autor examina la respuesta inmunitaria del ganado frente al virus de la fiebre aftosa, tanto después de una infección natural como de una vacunación, y pasa revista a las características y propiedades de los virus de fiebre aftosa y las vacunas con respecto a la protección que confieren. Se presta una especial atención a posibles enfoques que pueden ser utilizados para la mejora de las vacunas convencionales o la producción de nuevas vacunas contra esta enfermedad de importancia capital.


REFERENCES


