CURRENT STATUS AND POTENTIAL OF FOOT-AND-MOUTH DISEASE VACCINES

Fred Brown
USDA, Plum Island Animal Disease Center
P.O. Box 848
Greenport, NY 11944

Introduction

Foot-and-mouth disease (FMD) is the most economically important viral disease of farm animals, not only because of the loss of productivity which follows infection, but also because of the trade embargoes imposed on a country in which the disease is present. The disease is now largely confined to less developed countries, but it would be unwise to consider that other countries are safe. Rapid and large-scale international movement of people, animals and foodstuffs means that there is an ever-present threat to countries which are currently free from the disease. The United States has not had an FMD outbreak since 1929, but until vaccines became available which could be applied on a large scale, Western Europe had tens of thousands of outbreaks each year. It was the successful application of the Frenkel tongue epithelium vaccine, followed by products made in tissue culture cells, which led to the control of the disease on that continent. In fact, there have been no reported outbreaks of the disease in Western Europe since 1989.

The current FMD vaccines are prepared by imine inactivation of virus harvests from either tongue epithelial fragments or baby hamster kidney tissue culture cells. Properly prepared and mixed with an aluminium hydroxide-saponin or oil adjuvant these vaccines are potent immunogens and a single inoculation is sufficient to elicit levels of neutralizing antibody which will protect cattle and swine against a challenge dose of 10,000 ID_{50} FMD virus injected directly into the tongue (for cattle) or foot (for swine), or against the naturally occurring disease. These vaccines, which are stored as concentrates in liquid nitrogen, have a shelf-life of at least 18 months and form the reserve bank to be used in an emergency in several countries including the USA.

If FMD vaccines are so effective, it is fair to ask why the disease has not been eradicated worldwide in the past 40 years. One of the reasons may be the existence of wild-life reservoirs in some countries. Moreover, the carrier state, in which animals recovered from the disease continue to harbor the virus for a considerable time, may have a role in perpetuating the disease. More important, however, is the fact that FMD virus occurs as seven distinct serotypes which are constantly varying antigenically, posing problems regarding the selection of the most suitable vaccine to be used in any particular outbreak. There are additional problems in controlling FMD in less developed countries, not least of which are the greater logistical problems associated with more difficult terrain and geographical conditions in general, making it difficult to maintain the cold-chain so that the potency of the vaccine is retained until it is inoculated. There is also the suspicion among many farmers that the vaccines are not safe, with the consequence that they are often purchased but not used. In view of the unequivocal scientific evidence which has emerged from Western Europe during the last decade that most of the outbreaks there have been caused by

improperly inactivated vaccines (1, 2) these suspicions may not be unfounded.

An important question, therefore, is whether we can make absolutely safe and effective FMD vaccines. The answer to the first part of the question is yes. It is the second part of the question which poses the problem - a problem which I believe can be solved through research of the kind being done at Plum Island and in other laboratories around the world. To describe this approach, it is first necessary to summarize the properties of the virus and the structural features which elicit the protective immune response.

The Virus and Virus-related Particles

Foot-and-mouth disease virus consists of a particle, 300 Å in diameter, made up of a molecule of single-stranded RNA (molecular weight 2.6×10^6) and 60 copies of each of four proteins (molecular weights about 24×10^3 for VP1, VP2 and VP3, and about 10×10^3 for VP4). In addition to infectious particles, in virus-infected cells there are empty particles of a similar size but devoid of RNA. These empty particles contain the same proteins except that VP4 and VP2 are covalently linked. Also present is a particle (the so-called 12S pentameric sub-unit) comprising five copies of each of VP1, VP2 and VP3 and a considerable amount of the viral RNA polymerase (also known as the virus infection associated [VIA] antigen).

Infectious virus particles and empty particles are immunogenic, whereas the 12S particle is weakly immunogenic and the VIA antigen is devoid of this activity. Structural relationships between these particles have allowed us to build a model which explains many of the immunogenic properties of the virus.

Approaches to New Vaccines

The first approach to a new-style vaccine stemmed from two observations. Firstly, it was shown that immunogenicity of the infectious virus particle of FMD virus serotype O was considerably reduced by treatment with trypsin (3). Trypsinisation resulted in cleavage of VP1 but the particle was otherwise unaltered. The second observation was that only VP1 of the four capsid proteins elicited any neutralizing antibody (4). These observations led to experiments at Plum Island to express VP1 in Escherichia coli cells (5). Although the level of expression was excellent, the genetically engineered product had low immunogenicity, probably because the three-dimensional folding of the protein molecule differed from that which it assumes when it forms part of the virus particle.

The second approach was based on the observation that the amino acid sequences of certain regions of VP1 varied considerably. Reasoning that antigenic variability of the virus would be reflected in amino acid sequence variability in the VP1 protein it was demonstrated that peptides corresponding to the highly variable 138-160 amino acid region of the VP1 molecule were immunogenic. One inoculation of 50 ug of such a peptide protected guinea pigs against challenge infection. By presenting the peptide on hepatitis B core particles or as a tetramer or octamer, protection can be obtained with much smaller amounts of peptide (6, 7). However, antibody responses in cattle and swine to the peptide alone are considerably lower than those in guinea pigs. The problem seems to be the lack of a T cell epitope on the peptide which is

appropriate for cattle and swine. However, preliminary work has shown that protection of swine can be achieved by presenting the peptide on the hepatitis B core particle, the latter presumably providing the necessary T cell help.

A third approach is being made which involves the biosynthesis of the empty particles referred to earlier. Empty particles which have been produced in E. coli cells and in the baculovirus Autographa californica are immunogenic in both guinea pigs and swine (M.J. Grubman, personal communication).

By inserting the gene coding for a sequence of amino acids, including the 138-160 VP1 region referred to above, into the genome of bovine rhinotracheitis virus, a hybrid virus has been obtained which elicits protective levels of neutralizing antibodies in calves (8). The success of this approach is clearly of importance but its application in the field will depend on the absence of immunity to the vector.

All of the approaches could form the basis of a vaccine which could not cause FMD, thus allaying suspicion regarding the safety of the product.

The Chemical Basis of Antigenic Variability

It has been known for many years that the specificity of the immune response of the host to an antigen depends on the shape of the B cell epitope. We showed several years ago that the amino acids at positions 148 and 153 of the 141-160 immunogenic peptide are of particular importance in determining the specificity of the response (9). With four viruses of serotype A which differed only at those positions in the entire capsid protein region, clear differences could be found in their cross-reactivity in the neutralization test. Extension of this analysis to four more viruses from the same source has confirmed that a single amino acid substitution in the immunogenic site is sufficient to alter the antigenicity of the virus. Moreover, analysis of the 141-160 peptides corresponding to these viruses by circular dichroism and nuclear magnetic resonance is providing a structural basis for the serologic observations (10, 11). This approach should provide us not only with the information to design synthetic vaccines of defined specificity but also with the opportunity to synthesize vaccines which will provide wider protection.

Conclusion

Knowledge of the structure of the virus at the molecular level has provided us with the opportunity to identify those regions of the particle which elicit a protective immune response. These regions can be synthesized chemically or biochemically, thus avoiding the use of the infectious virus particle and consequently any chance of causing infection. Moreover, an understanding of the structural basis for antigenic variation is being reached which should enable us to design more efficient vaccines.

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