Comparison between *in vitro* neutralization titres and *in vivo* protection against homologous and heterologous challenge induced by vaccines prepared from two serologically distinct variants of foot-and-mouth disease virus, serotype A22

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Guinea-pigs were challenged with homologous or heterologous strains of foot-and-mouth disease virus (FMDV) following vaccination with baby hamster kidney (BHK) monolayer cell-adapted or BHK suspension cell-adapted strains of FMDV serotype A22 Iraq 24/64. The protection afforded by these vaccines was analysed as a function of antigen dose and the *in vitro* serum virus neutralization titres achieved. The results show that the level of neutralizing antibody induced that afforded 50% protection was similar for both vaccines in homologous or heterologous challenge situations. However, although the dose of antigen required to achieve this titre against homologous virus was similar for the two vaccines, approximately 20-fold more of the suspension cell-adapted virus was required to elicit a protective titre against heterologous challenge compared to the dose of monolayer cell-adapted virus required. A synthetic peptide representing the amino acid sequence 135 to 167 of VP1, which is identical in the A22 Iraq 24/64 variant viruses, was shown to induce protection against both homologous and heterologous virus challenge.

Antigenic variation is an important feature of foot-and-mouth disease virus (FMDV; family *Picornaviridae*, genus aphthovirus). The virus occurs as seven serotypes and there is considerable antigenic diversity between viruses within each serotype. This antigenic variation has implications for the selection and use of vaccine strains, those which elicit antibodies capable of neutralizing a broad range of field viruses being preferred to viruses that induce responses of narrow specificity. For practical purposes the one-way antigenic relationships between virus strains are determined by *in vitro* neutralization assays using antivirus antisera and are expressed as $r$ values, which are the ratios of heterologous to homologous neutralization titres.

Following adaptation to growth in baby hamster kidney (BHK) 21 monolayer cells, the virus strain A22 Iraq 24/64 (Arrowsmith, 1975) elicits antibodies which neutralize a wide range of field strains of the A serotype (Rweyemamu et al., 1984). However, passage of this virus in BHK suspension cells, which are required for large-scale vaccine production and have a different karyotype from BHK monolayer cells (Capstick et al., 1966), leads to the selection of variants which induce antibody responses of only very narrow specificity (Bolwell et al., 1989a). Cell attachment assays suggest that the selective pressure resulting in these changes in properties of the virus is related to the poor ability of the monolayer cell-grown virus (MV) to attach to suspension cells (Bolwell et al., 1989a).

Cloned progeny from the MV clone 162-154, or suspension cell-grown virus (SV) clone 148-173 had essentially the same antigenic properties as the parent stocks from which they were derived, showing that these properties were not due to the presence of mixtures of antigenic variants. Furthermore, both monoclonal antibodies (MAbs) elicited by MV particles and polyclonal antisera elicited by synthetic peptides, representing the immunodominant sequence of VP1 amino acids 135 to 167 (Bittle et al., 1982) which is common to both viruses, neutralized a wide range of field strains indicating that broad spectrum antigenic properties can reside in a single epitope on the virus (Bolwell et al., 1989b).

The potential efficacy of a vaccine is determined by two major factors, firstly by its immunogenicity in terms of the level of humoral antibody induced and, secondly, by the antigenic relationships between vaccine and field isolate strains. *In vitro* neutralization tests can provide valuable information on both these aspects. However, it is important to confirm predictions of vaccine efficacy based on *in vitro* tests with *in vivo* protection data. We have, therefore, examined the ability of vaccines prepared from MV, SV or the synthetic peptide VP1
Groups of guinea-pigs were inoculated with dilutions of purified (Brown & Cartwright, 1963) binary ethyleneimine (BED)-inactivated (Bahnemann, 1973) preparations of either the MV or SV viruses emulsified in Freund's incomplete adjuvant. For the SV vaccines animals received 5 µg (10), 1 µg (20), 0.2 µg (20) or 0.01 µg (20) of viral antigen intramuscularly and for the MV vaccine the doses were 5 µg (20), 1 µg (20), 0.2 µg (30), 0.04 µg (10) or 0.008 µg (10) (figures in parentheses denote the number of animals per group). At 43 days post-vaccination half of the animals in each group were challenged with homologous virus (MV or SV) and the other half were challenged with the heterologous virus A Bangladesh 8/78 by the method described by Black et al. (1985) and Francis et al. (1985). The relationship between antigen dose and percentage protection is shown in Fig. 1(a) and 1(b). Statistical analysis of the data (Cox, 1989; Williams, 1986) indicated that the dose of antigen required to protect 50% of the animals (PD50) against homologous challenge was similar for the two vaccines, equating to 0.05 µg for MV and 0.08 µg for SV (Table 1). A marked difference between vaccine groups was observed, however, when the animals were challenged with the heterologous virus, 50% protection being achieved with 0.014 µg of MV compared to 0.32 µg of SV (Table 1).

Sera collected 1 day prior to challenge in the above experiment were tested for their neutralizing activity against the challenge viruses by a two-dimensional microneutralization assay (Rweyemamu et al., 1978). Sera from animals vaccinated with MV neutralized both the homologous and heterologous viruses with equal efficiency; the error bars of the neutralization titres against both viruses overlap throughout the antigen dose range (Fig. 1c). However, sera from animals vaccinated with SV readily distinguished between the two viruses, the neutralization titres against the heterologous virus being significantly lower than those against the homologous virus (Fig. 1d). These results are in agreement with the r value determinations from earlier experiments (Bolwell et al., 1989a).

The protected status of individual animals against challenge with homologous or heterologous virus was

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**Table 1. 50% Protective dose (PD50) values**

<table>
<thead>
<tr>
<th>Vaccine</th>
<th>Challenge virus</th>
<th>Neutralization titre giving 50% protection</th>
<th>95% Confidence limits</th>
<th>Antigen dose (µg) giving 50% protection (PD50)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MV</td>
<td>MV</td>
<td>1.17</td>
<td>1.07 - 1.26</td>
<td>0.05</td>
</tr>
<tr>
<td></td>
<td>A Bangladesh</td>
<td>0.74</td>
<td>0.70 - 0.79</td>
<td>0.014</td>
</tr>
<tr>
<td>SV</td>
<td>SV</td>
<td>1.28</td>
<td>1.10 - 1.46</td>
<td>0.08</td>
</tr>
<tr>
<td></td>
<td>A Bangladesh</td>
<td>0.71</td>
<td>0.57 - 0.89</td>
<td>0.32</td>
</tr>
</tbody>
</table>
compared to the serum neutralization titre against the relevant virus at the time of challenge (Fig. 2a and b). There was generally good correlation between serum neutralization titre and protection, in agreement with earlier studies with FMDV (Pay & Hingley, 1986). The neutralization titres that correlated with 50% protection against homologous virus challenge were similar for MV and SV, being 1.17 and 1.28 log_{10} units respectively (Table 1). The titres giving 50% protection against heterologous virus challenge were also similar for MV and SV (Table 1). Surprisingly, however, they were significantly lower than the titres required for 50% protection against homologous challenge, being 0.74 and 0.71 log_{10} units respectively.

Since earlier work has shown that synthetic peptides representing the sequence of the G–H loop region of VP1 of A22 virus can induce cross-reactive neutralizing antibody responses (Bolwell et al., 1989b), the ability of peptide to induce protective responses has been investigated. Groups of 15 guinea-pigs were inoculated with either 25 µg or 250 µg of unconjugated peptide 135–167 Cys in Freund’s incomplete adjuvant. At 42 days post-vaccination, test bleeds were taken and five animals from each group were challenged with MV, SV or A Bangladesh 8/78 virus. The individual serum neutralization titres against the challenge virus and results of challenge are shown in Fig 2(c). Both dose levels of peptide induced good titres of neutralizing antibody against each of the viruses and only one animal was not protected. This single failure could not be correlated with the level of serum neutralizing antibody. Unfortunately the responses to even the lower dose of peptide were too high to determine the minimum weight of peptide or the minimum level of antipeptide neutralizing antibody required to induce protective immunity. However the results do emphasize the ability of peptide vaccines to induce broad protective immunity to virus challenge in this system.

The guinea-pig protection data reported here showed that, in the homologous challenge situation, the level of serum neutralizing activity which correlated with 50% protection was similar for both MV and SV. Furthermore the antigen doses required to elicit this level of response were also similar for both viruses. When animals vaccinated with MV or SV were challenged with a heterologous virus strain (A Bangladesh) the level of
serum neutralizing activity giving 50% protection was again similar for the two vaccines and, surprisingly, was somewhat lower than the titre affording protection against homologous challenge. However, the dose of antigen required to achieve this level of heterologous neutralizing activity was approximately 20-fold greater for SV compared to MV. Despite this, the high immunogenicity per unit mass of the SV variant compared to serotype O1 virus, for example, explains its efficacy as a vaccine strain in the field.

The distinct antigenic and immunogenic characteristics of MV and SV could not be correlated with amino acid substitutions within VP1. In fact, MV and SV have no sequence differences in the VP1 protein. Only three amino acid substitutions were found, all located in VP2 (Bolwell et al., 1989a) and, by comparison with the resolved structure of FMDV serotype O1 (Acharya et al., 1989), only two of these are predicted to be located at the surface of the virus. Despite this finding, the epitopes recognized by nine of 10 MAbs to MV, which have specificities similar to that of polyclonal antisera, clearly mapped to amino acids representing the G–H loop of VP1 (residues approx. 137 to 159) (Bolwell et al., 1989b).

Furthermore, synthetic peptides representing this sequence have previously been shown to elicit broadly cross-reactive neutralizing antibodies (Bolwell et al., 1989b) and have been shown here to provide protection against homologous and heterologous challenge. Unfortunately the doses of peptide used to vaccinate the animals were too high to allow a quantitative analysis of the amount required to afford protection.

An indication of the molecular nature of the profound antigenic differences between MV and SV may be provided by the recent serological and crystallographic analysis of antigenic variants of serotype O1 virus selected with MAbs (Parry et al., 1990). In that case serological experiments suggested that the VP1 G–H loop region was involved in the antigenic differences between the parental and variant viruses, but sequence data showed that the single substitution in each variant which conferred the difference was situated in the B–C loop of VP1 (residue 43, 48 or 59). However, crystallographic analysis showed that these substitutions induced profound conformational changes in the VP1 G–H loop and it was concluded that the altered antigenic properties of the variant viruses were related to the changed orientation of this feature. The amino acid differences between MV and SV are all in VP2 and two of these (amino acids 82 and 88) are predicted to underlie the G–H loop of VP1 in one of its conformations by analogy to the O1 virus structure (Parry et al., 1990). Also, substitutions at position 80 of VP2 have been implicated in the resistance of A10 virus to neutralization by MAbs (Thomas et al., 1988). It is therefore possible that the substitutions at these positions affect the conformation and/or orientation of the VP1 G–H loop and thus affect the antigenic properties of the viruses. Such a possibility may also explain the differences in the cell attachment properties of MV and SV (Bolwell et al., 1989a) since an important component of the receptor binding domain of FMDV is also located in the G–H loop of VP1 (Fox et al., 1989; Surovui et al., 1989; Baxt et al., 1990). X-ray crystallographic analysis of MV and SV should help to resolve the precise details of the structural differences between the two viruses which account for their distinct biological properties.

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References


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