Effect of Trypsin Treatment on the Antigenic Characteristics of Plaque Variants of Type O and Type Asia-1 Foot-and-Mouth Disease Viruses

(Accepted 26 June 1978)

SUMMARY

Antigenic differences were demonstrated between the large and small plaque variants of both types O and Asia-1 foot-and-mouth disease viruses. Treatment of the large and small plaque variants of the viruses with trypsin essentially abolished the observed antigenic differences. Thus, these plaque variants have antigenically different trypsin-sensitive determinants that may influence their immunogenicity and infection capabilities.

Large plaque (LP) and small plaque (SP) variants of type A (Cowan, 1969), type O (Dinter et al. 1959; McVicar & Sutmoller, 1972a, b), and type Asia-1 (Cowan et al. 1974) foot-and-mouth disease (FMD) viruses have been demonstrated to have different antigenic characteristics. It was further found that the plaquing and antigenic characteristics of Asia-1 virus produced in cell cultures were determined by the particular baby hamster kidney cell line (BHK-21, clone 13 of MacPherson & Stoker, 1962) used for virus passage, the culture method applied, i.e. monolayer or suspension culture, and the passage history of the virus (Cowan et al. 1974). Meloen (1976) reported a similar finding with type O1 virus, where virus grown in BHK suspension culture was considered antigenically deficient when compared with virus grown in suspended fragments of bovine tongue epithelium (Frenkel, 1950). Although the plaque characteristics of these viruses were not indicated, our experience would lead us to expect that the Frenkel-grown virus would be a LP former while the BHK suspension virus would be a SP former. Meloen (1976) made the additional interesting observation that the 'antigenic deficiency' of BHK suspension passaged virus was attributable to the trypsin-sensitive (TS) site on the virion. The present report confirms Meloen's observation, but further shows that SP variants are not deficient in the TS antigenic determinant, but that the SP and LP variants have antigenically different TS determinants.

Type O1 plaque variants were derived from the 1969 Turkish field strain, Manisa, as described by Erol et al. (1975). The original field isolate and subsequent cattle passages were found to produce mainly plaques of approx. 1 mm diam. and 1 to 3% plaques of 3 to 6 mm diam. when assayed on BHK-21 (Razi line) monolayer cultures overlaid with methylcellulose (Tylose) (Cowan et al. 1974). Passage of this virus in BHK-21 (Razi line) monolayer roller bottle cultures did not result in any modification of the plaque character of the virus, i.e. it remained predominantly a SP former.

Passage of the original virus in Frenkel culture resulted in a rapid change to a virus population that produced large plaques (3 to 6 mm diam.) as described by Erol et al. (1975). Only three to five passages of the virus in Frenkel culture were required to obtain virus that was almost solely LP in character. The LP character of the virus was retained after at least six passages in BHK-21 (Razi line) monolayer cultures. The LP and SP type O1 viruses were grown in roller bottle monolayer cultures of Razi line BHK-21 cells and concentrated...
by precipitation with polyethylene glycol (PEG; Carbowax 6000) as described previously (Wagner et al. 1970; Cowan et al. 1974).

The preparation and characterization of the LP and SP variants of type Asia-1 virus have been described (Cowan et al. 1974). Concentrated preparations of these viruses were prepared as indicated previously.

Antisera to LP and SP variants of type O1 virus were prepared in cattle by intradermal inoculation of the desired virus (Erol et al. 1975). Cattle inoculated with the original virus (predominantly SP in character) were severely infected with extensive lesions of the tongue and feet. Virus isolated from these lesions retained the SP character. The cattle were bled 7 days post inoculation (p.i.) and the serum was used for subsequent immunological analyses. Cattle were also inoculated with the LP variant but only minor lesions occurred on the tongues and there was no evidence of generalization to the feet. Blood was collected at 7 days p.i. to provide serum.

Antisera to the LP and SP variants of type O1 virus were also produced in guinea pigs by intradermal inoculation into the rear foot-pads. Both variants produced lesions, but those produced by LP virus were less severe (Erol et al. 1975). Blood was collected from some of each group of animals at 7 days p.i., and the other animals were kept for about 35 days before being bled. Antisera to type Asia-1 SP and LP variants were prepared in guinea pigs in a similar manner. The sera were stored at -20 °C and heated for 30 min at 56 °C before use.

Virus preparations were treated with trypsin according to the general procedure of Wild & Brown (1967), but PEG-concentrated preparations rather than purified viruses were necessarily used. Briefly, the samples were treated with 1.0 mg of trypsin (Difco, 1:250) per 0.8 ml of sample for 15 min at 37 °C and normal guinea pig serum added to stop the action of the trypsin. The untreated virus preparations also received normal guinea-pig serum for control purposes. The reagents and procedures used for the agar gel immunodiffusion reactions have been described (Cowan et al. 1974).

Immunodiffusion tests were performed with the plaque variants of both type O1 and type Asia-1 viruses against the different antisera with similar results. The 7-day p.i. sera demonstrated antigenic differences more clearly than sera collected later, which is in accord with the findings of others (Cowan, 1969; McVicar & Sutmoller, 1972b; Meloen, 1976). Consequently, only reactions with the early sera will be considered.

The general findings when type O1 LP and SP viruses were tested against their respective bovine antisera are illustrated in Fig. 1(a). When tested against anti-LP serum, LP virus produced a precipitin band that spurred over that obtained with SP virus, and, in turn, the anti-SP serum resulted in precipitin bands where SP virus spurred over LP virus. Thus, the LP and SP viruses may be considered to contain common antigenic determinants, but each also carries distinctively different ones. When this precipitin test was repeated with trypsin-treated virus preparations (Fig. 1b), the spurs were essentially eliminated and coalescing precipitin bands appeared to be obtained. Thus, trypsin destroyed the antigenic determinants on both LP and SP viruses which were primarily responsible for their antigenic differentiation.

The antigenic relationships between the various virus preparations were examined with additional immunodiffusion reactions (Fig. 2a, b). With bovine anti-SP serum in the central well (Fig. 2a), SP virus spurred strongly over both trypsin-treated SP (SP-T) and LP virus preparations. Although it is not clearly evident in the photograph, SP-T produced very faint spurs over both LP and LP-T virus preparations indicating a slight difference in LP and SP viruses other than that related to the TS determinants. A similar conclusion can be
short communications

439

Fig. 1. Immunodiffusion reactions of bovine antisera to large plaque (a-LP) and small plaque (a-SP) variants of type O1 foot-and-mouth disease virus with (a) untreated large plaque (LP) and small plaque (SP) type O1 viruses, and (b) with trypsin-treated (SP-T and LP-T) virus preparations.

drawn from results presented in Fig. 2 when bovine anti-LP serum was tested against the different antigen preparations. In this instance, the LP virus precipitin band spurred strongly over those formed by SP, SP-T and LP-T preparations. The SP and SP-T precipitin bands coalesce, but LP-T produced a faint spur over SP-T. These observations were confirmed with guinea-pig antisera which, in fact, exaggerated some of the differences cited.

Meloen (1976) suggested that type O1 FMD virus grown in BHK-21 suspension cultures was deficient in the TS antigenic determinant when compared with the virus grown in Frenkel cultures. We may well have drawn the same conclusion if we had not had antisera to the different plaque variants that were examined in this study. Using these antisera leads us to conclude that neither plaque variant is deficient in the TS antigenic determinant, but that they are antigenically different.

Foot-and-mouth disease virus contains four major capsid proteins: VP1, VP2, VP3 and VP4 (LaPorte, 1969; Wild et al. 1969; Vande Woude et al. 1972; Strohmaier & Adam, 1974) only one of which is sensitive to trypsin (VP1 in the terminology of Burroughs et al. 1971; LaPorte et al. 1973; Strohmaier & Adam 1974, and VP2 in the case of Bachrach et al. 1975). The TS peptide is of major interest because it is primarily responsible for immunogenicity (Wild & Brown, 1967; LaPorte et al. 1973; Bachrach et al. 1975; Cavanagh et al. 1977; Kaaden et al. 1977). Consequently, virus variants having antigenically different TS peptides may be expected to differ in their immunizing ability when used in vaccine preparation. Their relative efficacy would reasonably be related to the antigenic nature of the TS peptide on the virus used to challenge the immunity of animals receiving such vaccines. That such is the case was indicated in a study in which the efficacy of type Asia-1 vaccines prepared from LP and SP virus variants was compared (Cowan et al. 1974). Vaccine prepared from the LP variant was more effective than that prepared from the SP variant when the challenge virus (field virus) had LP characteristics. In this regard it is of interest to note
that the type O1 field virus was small plaque in nature, but rapidly changed to large plaque on passage in Frenkel culture. This would suggest that the plaque characteristics of viruses responsible for outbreaks should be performed directly on field samples and not on passaged samples adapted to laboratory propagation systems. Otherwise one could be misled on the plaque and antigenic character of the virus responsible for the outbreak.

An additional vital role of the TS peptide of the virion concerns its identification as the specific site required for attachment of the virion to susceptible cells (Wild & Brown, 1967; Cavanagh et al. 1977). Antigenic differences in the TS peptide of virus variants indicate chemical or conformational differences or both, and these differences could influence their ability to attach to and infect different types of cells. An early study by Diderholm & Dinter (1965) showed that polyoma-transformed BHK-21 cells were less susceptible to infection by virulent FMD virus than to infection by attenuated strains of FMD virus. Furthermore, Bengtsson et al. (1963) found that dextran sulphate did not affect the plaque size of virulent virus, but it markedly decreased the plaque size of attenuated FMD viruses. Thus, the plaque size of FMD viruses plated in the presence of dextran was related to virulence as was the ability of virulent and attenuated viruses to infect transformed BHK-21 cells.

Results in studies (Cowan et al. 1974) with plaque variants of type Asia-1 virus were similar in that the LP variant was unable to infect BHK-21 cells highly adapted to suspension, whereas the SP variants readily infected the highly transformed cells as well as untransformed or cells newly adapted to suspension.

The role of the FMD virus TS peptide in immunogenicity and in determining infectivity may be of vital concern in the preparation of satisfactory vaccines. Variants with different TS peptides may differ antigenically as well as in their ability to attach to and replicate in different lines of BHK-21 cells grown under different conditions. An earlier caveat (Cowan

Fig. 2. Immunodiffusion reactions of type O1 foot-and-mouth disease virus large plaque (LP), trypsin-treated large plaque (LP-T), small plaque (SP) and trypsin-treated small plaque (SP-T) preparations with (a) bovine anti-small plaque serum (a-SP) and (b) bovine anti-large plaque serum (a-LP).
et al. 1974) concerning the antigenic characteristics of virus variants, the BHK-21 cell lines used for virus growth, and culture methods applied still appears appropriate.

Plum Island Animal Disease Center
USDA, SEA, NER
P.O. BOX 848
Greenport, New York 11944, U.S.A.

References


* Present address: VRD, USDA, SEA, P.O. Box 32, Kikuyu, Kenya.
Short communications


(Received 9 March 1978)