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Immune response to virus-infection-associated (VIA) antigen in cattle repeatedly vaccinated with foot-and-mouth disease virus inactivated by formalin or acetylethyleneimine

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SUMMARY

The results of experiments to investigate antibody to 'virus infection associated' (VIA) antigen in cattle repeatedly vaccinated with formalin- or acetylethyleneimine- (AEI) inactivated foot-and-mouth disease (FMD) vaccines under laboratory conditions are reported. Results are also presented from some vaccinated animals subsequently exposed to FMD infection.

Antibody against VIA was not detected before and after the first vaccination with formalin or AEI-inactivated vaccine but did develop in all animals after the second formalin vaccination and persisted throughout the experiment. After the second AEI vaccination, 4 of 12 animals developed antibody which persisted for at least 37 days. This transient response in some cattle was repeated after successive vaccinations but, in general, more animals responded as the number of vaccinations increased.

After exposure to infection, a transient VIA antibody response was occasionally observed in immune AEI-vaccinated animals. Some immune repeatedly AEI-vaccinated cattle did not develop detectable VIA antibody after challenge despite the persistence of virus in oesophageal–pharyngeal (O/P) fluid.

The presence of antibody to VIA antigen is not conclusive proof that vaccinated animals have been exposed to infection and field data must be interpreted with caution.

INTRODUCTION

A group-specific antigen occurring in tissues infected with foot-and-mouth disease virus (FMDV) was described by Cowan & Graves (1966). It appeared to be a non-structural virus component produced in the course of infection and was therefore named 'virus-infection-associated' (VIA) antigen. However, Rowlands, Cartwright & Brown (1969) reported evidence indicating that this antigen may occur as a structural virus component.

Antibody against VIA was originally demonstrated only in sera from animals infected with FMDV and not in sera from animals immunized with inactivated vaccines (McVicar & Sutmöller, 1970). These authors also reported that the double

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immunodiffusion (DID) test for VIA antigen provided a rapid and accurate means of distinguishing animals which had been infected from those which had not and suggested the use of the test in epizootiological investigations.

It has since been shown that cattle vaccinated with virus inactivated with formalin but not with acetylethyleneimine (AEI) can give a positive reaction to VIA antigen attributed either to the multiplication of incompletely inactivated virus or to the presence of VIA antigen in fluids from infected tissue cultures used to formulate the vaccine (Fernandez *et al.* 1975).

More recently, Dawe & Pinto (1978) reported a transient response to VIA antigen in cattle vaccinated twice with AEI inactivated vaccine under field conditions.

In this report we describe the results of experiments carried out in cattle vaccinated up to six times with formalin or AEI inactivated vaccine under strictly controlled laboratory conditions.

MATERIALS AND METHODS

Foot-and-mouth disease virus (FMDV) of type A (A_{24} Cruzeiro) was used to prepare VIA antigen. The virus was grown in monolayers of baby hamster kidney cells (BHK 21: Macpherson & Stoker, 1962).

VIA antigens were prepared by a modification of the methods described by Cowan & Graves (1966) and Lobo, Gutierrez & Marino (1974). Dry Sephadex A-50 was added to virus harvests to a concentration of 1 g of gel per 1·5 l of clarified harvest or to the supernatant after ultracentrifugation to remove 140S material. The mixture was stirred for 2 days at 4 °C and the slurry poured into a chromatography column. The 140S and 12S components were eluted with 0·15 M-NaCl in 0·02 M tris buffer at pH 7·6 and subsequently VIA antigen was eluted, using the same buffer system with sodium chloride at 1·0 M. The effluent was monitored by ultraviolet spectrophotometry at 280 nm. The peak fractions containing VIA antigen were concentrated by negative pressure dialysis against PBS at pH 7·6.

The identity and purity of VIA antigen was tested by the double immunodiffusion (DID) test against positive sera.

VIA antigens prepared from harvests of all seven immunological types of FMDV (O_1 BFS 1860, A_{24} Cruzeiro, C-CGC, SAT 1/Malawi 3/70, SAT 2/Tanzania 2/75, SAT 3/Malawi 8/76, and Asia 1/Pakistan 1/54) gave reactions of complete identity when compared with each other by immunodiffusion.

VIA antigen was also prepared from FMDV type O (O_1 BFS (1860) BHK suspension cultures after inactivation with AEI. The cells were sedimented and the supernatant fluid was concentrated 16-fold by ultracentrifugation on a zonal gradient in 10 to 45% sucrose. Approximately 200 ml of suspension were collected from the top of the gradient in the region corresponding to 10% (w/v) sucrose. VIA antigen was readily obtained from this suspension by chromatography according to the procedure described above.

Double immunodiffusion (DID) test

The tests were carried out as described by Pinto & Hedger (1978) in 85 mm disposable plastic Petri dishes containing 16 ml of 1% Oxoid agar No. 2 in 0·02 M tris buffer with 0·15 M-NaCl and 0·1% sodium azide at pH 7·6.

Following the criteria of McVicar & Sutmöller (1970), the development of a discernible precipitin band was considered positive if an identity reaction with the positive control band was evident. Reactions in which the end of the positive control band clearly curved away from the test serum well were classified as weakly positive. When the positive control band ran straight into or very close to the test serum well, the reaction was considered negative.

Virus isolation and serum neutralization tests

Virus isolation from oesophageal-pharyngeal (O/P) fluid was made on primary bovine calf thyroid cells grown in tubes according to the technique previously described by Snowdon (1966).

Serum neutralization tests (SNT) were performed using the microtitre method described by Golding *et al.* (1976).

Vaccines

Cattle were immunized with vaccines prepared by the Wellcome Foot-and-Mouth Disease Laboratory, Pirbright. The vaccines were prepared from various types of FMDV as specified below. Viruses were grown in BHK cell suspension culture, filtered and inactivated with formalin or AEI. Saponin and alhydrogel were incorporated as adjuvants. The vaccines were administered by subcutaneous or, in the case of Group 2, by subcutaneous or intravenous inoculation, as shown in Table 2.

Vaccination and sampling

The experiments were carried out in Devon steers, 18–24 months old, housed in disease-secure isolation units. Sequential serum samples were obtained before and after vaccination. Three groups of animals were studied:

Group 1. Twelve steers vaccinated up to four times with a formalin inactivated bivalent type O-A vaccine, as shown in Table 1.

Group 2. Twelve steers vaccinated up to six times with one of two AEI inactivated bivalent type O-A vaccines, as shown in Table 2.

Group 3. Twenty-four steers vaccinated in groups of 3 animals (groups A to H) vaccinated once, twice or three times with AEI inactivated type C (strain Noville) FMD vaccine at varying intervals before exposure to infection, as shown in Table 3. Samples of O/P fluid were obtained from each animal for 10 days after exposure to infection and at irregular intervals thereafter up to 21 days.

Exposure to infection

Cattle in Group 3 were exposed to infection by holding groups of three steers for 1 h in a loose-box immediately after it had been vacated by 6 donor pigs

Table 1. *Antibody response to VIA antigen in cattle repeatedly vaccinated with formalin inactivated FMD vaccine*

Times vaccinated	Days after vaccination and revaccination at the time of sampling							
1	0*	14	21	28	113	124	133	149
2	.	.	.	.	0*	11	20	36
3	.	.	.	.	.	0*	9	25
4	.	.	.	.	.	.	0*	16
VIA antibody positive / No. animals sampled	0/12	0/12	0/12	0/12	0/12	12/12	12/12	12/12

* Day of vaccination or revaccination.

infected with type C (strain Noville) FMDV 48 hours previously. Recipient groups were returned to uninfected boxes and the donor pigs were slaughtered after the final exposure period.

RESULTS

Group 1

The VIA antibody results from cattle vaccinated up to 4 times with a formalin inactivated vaccine are summarized in Table 1. Antibody against VIA antigen was not detected before and after the first vaccination. Eleven days after the second vaccination all animals had developed antibody against VIA antigen and the antibody was consistently detected in all subsequent samples.

Group 2

Antibody results from cattle vaccinated up to six times with AEI inactivated vaccine are summarized in Table 2. Antibody against VIA antigen was not detectable before or after the first vaccination. However, 7 days after the second vaccination four animals developed VIA antibody which persisted in samples taken 14 and 37 days later but was no longer detectable after 85 days. This pattern of a transient response to VIA in some members of the group was repeated after successive vaccinations but, in general, more animals responded as the number of vaccinations increased.

Group 3

The vaccine stimulated satisfactory titres of serum neutralizing antibody at the time of exposure in all animals except in groups A and B. The geometric group mean antibody titres of exposure were: group C 2·0; D 1·9; E 2·0; F 2·7; G 3·2; H 3·0. The animals were protected against the development of clinical disease except in groups A and B, in which all cattle developed fully generalized disease.

The results of tests for VIA antibody before and after exposure and of tests for O/P virus after exposure of cattle to FMDV are summarized in Table 3.

Antibody to VIA antigen was not detected before exposure to infection in cattle vaccinated only once but did appear in four animals vaccinated twice or three times in groups F and H.

After exposure, virus was detected in O/P samples from all but two animals. All three animals vaccinated at the time of exposure (group A) developed anti-

Table 2. *Antibody response to VIA antigen in cattle repeatedly vaccinated with AEI inactivated FMD vaccine*

Animal no.	Times vaccinated and route	Days after vaccination or revaccination at the time of sampling													
.	1 s.c.	0	7	14	21	28	35	58	106	121	150	162	197	204	213
.	2 s.c.	.	.	.	0*	7	14	37	85	100	129	141	176	183	192
.	3 s.c.	.	.	.	.	.	.	.	.	0*	29	41	76	83	92
.	4 s.c.	.	.	.	.	.	.	.	.	.	0*	12	47	54	63
.	5 i.v.	.	.	.	.	.	.	.	.	.	.	0*	35	42	51
.	6 s.c.	.	.	.	.	.	.	.	.	.	.	.	.	0*	9
1	.	−	−	−	−	W	−	−	−	−	−	+	W	−	+
2	.	−	−	−	−	−	−	−	−	−	+	−	−	−	−
3	.	−	−	−	−	−	−	−	−	−	+	W	+	−	−
4	.	−	−	−	−	−	−	−	−	−	−	+	+	W	+
5	.	−	−	−	−	−	−	−	−	−	−	−	−	−	−
6	.	−	−	−	−	−	−	−	−	−	+	−	−	−	−
7	.	−	−	−	−	+	+	W	−	−	+	+	+	W	+
8	.	−	−	−	−	−	−	−	−	−	−	+	−	W	+
9	.	−	−	−	−	−	−	−	−	−	+	−	W	−	−
10	.	−	−	−	−	+	+	+	−	−	−	+	−	W	+
11	.	−	−	−	−	+	+	+	−	−	−	−	−	−	−
12	.	−	−	−	−	NS	+	NS	−	−	NS	−	NS	−	−
Strong positive		0/12	0/12	0/12	0/12	3/11	4/12	2/11	0/12	0/12	5/11	5/12	3/11	0/12	5/12
Weak positive		0/12	0/12	0/12	0/12	1/11	0/12	1/11	0/12	0/12	0/11	1/12	2/11	4/12	0/12
Total positive		0/12	0/12	0/12	0/12	4/11	4/12	3/11	0/12	0/12	5/11	6/12	5/11	4/12	5/12

* Day of vaccination or revaccination. + = strong positive reaction. W = weak positive reaction. − = negative reaction. NS = no sample available. s.c. = subcutaneous route. i.v. = intravenous route.

Table 3. *Summarized results of virus isolation from oesophageal–pharyngeal fluid and antibody to VIA antigen in cattle vaccinated up to three times at varying intervals before exposure*

	Vaccination schedule*			Oesophageal–pharyngeal virus		VIA antibody		
						Animals positive		
Group	× 1	× 2	× 3	Animals positive†	Samples positive / Samples taken‡	Before exposure	After exposure	Samples positive / Samples taken§
A	0	.	.	3	26/30	0	3	11/36
B	7	.	.	3	24/30	0	3	17/36
C	14	.	.	3	29/30	0	1	2/36
D	21	.	.	3	19/30	0	1	3/36
E	28	0	.	3	16/30	0	2	14/36
F	35	7	.	3	17/30	3	3	30/36
G	42	14	.	3	19/30	0	2	4/36
H	414	379	14	1	3/30	1	1	10/36

Cattle in groups A and B developed disease after exposure; those in groups C–H were clinically immune.

* Days vaccinated or revaccinated at time of exposure.

† Number of animals from which O/P virus was isolated in each group of 3 animals.

‡ Number of samples from which O/P virus was isolated/(number of animals sampled × number of samples taken). $n/(3 \times 10)$ samples taken during the first 10 days after exposure.

§ Number of samples positive for VIA antibody/(number of animals sampled × number of samples taken). $n/(3 \times 12)$ samples taken during the period up to 42 days after exposure.

body against VIA by day 8–10 after exposure, while those vaccinated 7 days before exposure (group B) showed antibody to VIA by day 5 in two animals and by day 9 in the other.

Single animals in groups C and D showed an antibody response to VIA. The remaining animals in each of these groups were negative. In these animals, virus multiplication as shown by O/P virus recovery was coincident with high levels of virus-neutralizing antibody in the serum.

In group E one animal was negative and the two others positive to VIA by day 5 and day 7 after exposure.

In group F three animals developed antibody against VIA one day before exposure; one of them gave a weak positive result on that day, becoming positive one day after exposure. All animals maintained this antibody throughout the sampling period after exposure.

In group G two steers developed VIA antibody by day 14 after exposure; one showed a positive reaction on that day but the reaction became weakly positive by day 21. No VIA antibody was detected in the other steer.

In group H viral recovery was confined to a single animal and detected on only three occasions during the first 10 days of sampling and in 3 out of 10 subsequent samples taken at intervals during the period 10–42 days after exposure. However, virus was recovered from pharyngeal tissue samples taken from all 3 animals in this group at post-mortem 42 days after exposure. Two animals did not develop antibody to VIA whereas the third, the animal from which virus was detectable

in O/P samples, developed antibody before exposure which decreased below detectable levels within 14 days after exposure.

DISCUSSION

VIA antigen is produced during viral replication (Cowan & Graves, 1966; Rowlands *et al.* 1969) and it follows that culture fluid harvested for vaccine preparation will contain this component. In routine vaccine preparations the antigen may not be present in sufficient quantity to be easily detectable or to stimulate antibody formation in cattle. However, during the course of this study, VIA antigen was readily demonstrated in the supernatant of vaccine cultures inactivated with AEI and concentrated by zonal centrifugation.

McVicar & Sutmöller (1970) and Fernandez *et al.* (1975) demonstrated the presence of VIA antibody in cattle vaccinated with formalin-inactivated vaccine. They drew attention to the fact that vaccines so produced may contain residual infective virus (Wesslén & Dinter, 1957; Fellowes, 1960; Graves, 1963) and that limited replication of this virus could produce an immune response to VIA antigen in the absence of clinical disease. However, the fact that antibody to VIA was observed in our study only after the second vaccine dose (Table 1) suggests that it resulted from a recall response to antigen present in the vaccine rather than to residual virus multiplication, which would be more likely to occur in unvaccinated animals.

In these experiments VIA antibody was more often detected in cattle immunized with formalin than with AEI vaccines. Inactivation using AEI follows first-order kinetics and complete inactivation is reliably obtained (Brown *et al.* 1963). The efficacy of AEI together with stringent innocuity testing in tissue culture renders it extremely unlikely that such vaccines will contain residual live virus.

However, an alternative explanation may be that VIA antigen is better preserved by treatment with formalin than with AEI. In fact, the reaction of semi-purified VIA antigen with antibody has been shown to be considerably reduced after incubating the antigen for 6 hours at 37 °C with 0·05% AEI, whereas a much smaller loss of activity was observed when the antigen had been treated with 0·05% formaldehyde for 72 h at room temperature (D. J. Rowlands, unpublished results).

The experiments confirm the report of Fernandez *et al.* (1975) in that VIA antibody was not detected after a single application of AEI saponin-alhydrogel vaccine. In animals vaccinated two or more times with AEI vaccine (Tables 2 and 3) a transient antibody response to VIA appeared, showing that repeated immunization may result in antibody formation, probably as a cumulative response to VIA antigen present in vaccine. Antibody persisted for at least 37 days after the second vaccination but was no longer detectable after 85 days (Table 2). Dawe & Pinto (1978), using a similar vaccine in the field, found VIA antibody in 14 of 51 annually vaccinated cattle 3 weeks after re-immunization, with 5 of the animals positive 2 weeks later. It is possible that the quality and quantity of VIA antigen normally present in AEI-inactivated FMD vaccine is on the threshold of

effective immunogenicity so that the response is variable and short-lived. Indeed, the pattern following successive vaccinations is compatible with a repeated IgM response which is not converted to give an IgG component.

No correlation could be drawn between the serum neutralizing antibody titre, virus detection in O/P fluid and the detection of VIA antibody, which confirms the findings of Fernandez *et al.* (1975) for 12 infected cattle. However, a survey on 432 sera from buffalo (*Syncerus caffer*) showed that animals with high specific serum antibody titres were more likely to be positive to VIA than those with low levels (Pinto & Hedger, 1978). The difference may be attributable to the number of animals tested and the relative time interval between exposure and sampling, which was probably much longer in the buffalo study.

A striking observation was apparent in groups C, D and E (Table 3), where 5 of 9 animals were negative for VIA antibody after exposure despite the repeated recovery of virus from pharyngeal secretions. Carrier animals do not therefore invariably develop VIA antibody detectable by the immunodiffusion test. It is possible that some VIA-positive animals might have become negative if sampled over a longer period.

Lobo *et al.* (1976) reported that the VIA antibody response following exposure of cattle which had received seven or more applications of formalin-alhydrogel vaccine was similar to that of unvaccinated cattle and concluded that repeated vaccination of this type does not interfere with the development of VIA antibody. The VIA antibody status of the animals before exposure was not reported. In our study, repeated vaccination was associated with a decrease in the time for which VIA antibody could be detected. For example, antibody could no longer be detected in two animals from groups G and H by days 14 and 21 after exposure. This could be explained by the fact that highly immune animals have humoral and secretory antibody at titres which markedly reduce the replication and excretion of infective virus (Garland, 1974), so that the antibody response to VIA antigen could be diminished as a consequence. The results of McVicar & Sutmöller (1970) show such an effect in that positive VIA reactions in AEI-vaccinated cattle reached a peak of 50% at 2 weeks after exposure and fell to 37 and 11% at 3 and 4 weeks after exposure, respectively. Additional evidence of the effect of high levels of immunity was provided by the marked reduction in the amount of virus recoverable in O/P fluid from thrice-vaccinated steers in group H, where virus and VIA antibody were detected in only 1 of 3 animals. These findings were investigated further at post-mortem 42 days after challenge. Although no virus could be demonstrated in O/P fluid samples taken immediately before slaughter, virus was recovered in samples from the dorsal surface of the soft palate of all three steers (Garland, 1974). However, in the only animal in this group in which VIA antibody was detected, on the day before exposure, the VIA response was transient and could not be detected beyond day 14 after exposure.

It must be noted that the regimens used in these studies involved much more frequent vaccination than is normally found in the field. Nevertheless, the studies show the wide variation in the VIA response of individual animals after repeated vaccination and challenge.

The studies show the wide variation in the VIA response of individual animals after repeated vaccination and challenge. VIA antibody may or may not develop after repeated immunization but the frequency and duration of the response appears to be greater after formalin vaccination than AEI vaccination. The results emphasize the need for caution in the interpretation of tests for VIA antibody. Ideally, field surveys should also include appropriate tests for serum neutralizing antibody and for virus carriers. The epizootiological situation in the sampling area, the nature, frequency and exact timing of any vaccination, the age of the animals sampled and the status of the dam when young stock are sampled can all influence the occurrence of VIA antibody. Detailed information of this kind is often not obtainable with certainty in the field but without it the interpretation of VIA antibody results could well be misleading.

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