INNOCUITY CONTROL OF ALUMINUM-HYDROXIDE SAPONIN FOOT- AND - MOUTH DISEASE VACCINES BY ELUTION AND CONCENTRATION OF THE ANTGEN

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SUMMARY

A method of innocuity control of inactivated aluminum-hydroxide saponin foot-and-mouth disease vaccines is described. The antigen was eluted from the aluminum-hydroxide by a Matheka buffer followed by concentration with polyethylene glycol and inoculation of BHK cell cultures in Roux bottles. With each series of vaccine, 1 ml of the eluted and concentrated antigen was inoculated in each of three Roux bottles which represents a total of 150 ml of vaccine.

INTRODUCTION

Innocuity controls of inactivated aluminum-hydroxide saponin foot-and-mouth disease (FMD) vaccines are performed in cattle (6), suckling mice (7) and in cell cultures (1, 3 and 4). Presently, even though the cattle test is considered as the reference assay (6), cell cultures are more widely used, with preference of the inoculation of eluted antigen instead of the whole vaccine (3, 5 and 9), that usually contains substances toxic to the cells.

This paper describes the results of tests done to detect infectious virus in aluminum-hydroxide saponin FMD vaccines. In these experiments the vaccines were artificially contaminated with FMD virus, the antigen was eluted by a Matheka solution followed by concentration with polyethylene glycol (PEG) and inoculation of IBRS-2 and BHK₂¹ C 13 cells.

MATERIALS AND METHODS

Vaccine

The vaccines contained antigens produced in BHK₂¹ C 13 cells that were grown in suspension. The virus was inactivated with BEI (2) and adsorbed to aluminum-hydroxide. Also, each ml of final product contained one milligram of saponin.

Contamination of the vaccine

To 7 vials of vaccine a series of virus dilutions was added so that the bottles contained 10⁻⁰.², 10⁻¹.², 10⁻².², 10⁻³.², 10⁻⁴.², 10⁻⁵.² or 10⁻⁶.² infective dose 50% cell culture per ml (ID₅₀CC/ml), respectively. The contaminant strain used was C₃ Indaial-Brazil/71 adapted to BHK₂¹ C 13 cells. The contaminated vaccine was kept for 4 days at 4°C.

Elution and concentration of the antigen

The sediment obtained from 200 ml of each contaminated vaccines which was obtained by centrifugation at 1,000 g during 10 minutes at 4°C was resuspended in 20 ml of a Matheka solution (85 ml of 0.33M of Na H PO₄ 2H₂O and 15 ml of 0.33M KH₂PO₄, pH 7.5) (8). The mixture was lightly stirred for 45 minutes at 4°C, then centrifuged in the conditions previously mentioned and the supernatant was collected. The sediment was again resuspended in 20 ml of a Matheka solution for a second elution of the antigen. The 40 ml of the supernatant obtained with two treatments were mixed with 8% w/v of PEG 6000M. Mixture was again lightly stirred for 3 hours and then centrifuged at 4°C for 10 minutes at 8,000 g. The sediment was resuspended in 4 ml of Eagle medium without serum.

Inoculation of Roux bottles

One ml of the eluted and concentrated antigen was inoculated in each of three Roux bottles with monolayers 48-hour BHK₂¹ C 13 cells. If after
48-hour incubation at 37°C no cytopathic effect was observed, a second passage was made by the inoculation of a new Roux bottle with 10 ml of the cultures (after freezing and thawing). If no cytopathic effect occurred a third passage was made after 48 hours. All pools harvested of the three passages were examined by complement fixation.

**Tritration in microplaques**

The virus added to the vaccine, the supernatant of the contaminated vaccine, the eluted antigen and the concentrated antigens were titrated in cell cultures. Eight wells of microtiter plates containing 24 hours old monolayers of IBRS-2 C 17 cells were inoculated with 0.1 ml of a 10⁻¹ to 10⁻⁸ dilution series. The plates were covered and put in a CO₂ incubator at 37°C. After 48 hours of incubation, the plates were stained and fixed for 15 minutes with a 10% formalin solution containing 0.1% of violet crystal. All wells showing partial or complete cytopathic effect were considered positive. The 50% infective titer was calculated by the method of Spearman-Kärber and expressed as ID₅₀CC/ml.

**RESULTS**

Table 1 lists the infectivity titers/ml of the virus strain C₃ Indaiá-Brazil/71 of each of the 7 contaminated bottles (A, B, C, D, E, F, G). Also are listed infective titers/ml of the supernatant obtained by centrifugation of these vaccines and of the eluted antigens before and after concentration with PEG.

It was observed that 0.1 ml of the aqueous phase of the vaccine or of the antigen eluted with a Mathéka buffer were toxic for IBRS-2 cells up to the 10⁻¹ dilution. The whole vaccine also was toxic. However, 0.1 ml of the eluted antigen concentrated 10 times by PEG was not toxic for IBRS-2 cells grown in microtiter plates. Similarly, 1 ml of the antigen was not toxic for monolayers of BHK₂₁ C 13 cells grown in Roux bottles.

The eluted and concentrated antigen of the seven contaminated vaccines was inoculated in Roux bottles with monolayers of BHK₂₁ C 13 cells at 1 ml per bottle. All the vaccines were shown to be infectious at the first passage. The strain was typed at C₃ Indaiá.

**TABLE 1. Detection of active virus of infectious virus artificially contaminated with FMD virus strain C₃ Indaiá**

<table>
<thead>
<tr>
<th>Added to the vaccine (per ml)</th>
<th>In the vaccine supernatant</th>
<th>In the eluted antigen</th>
<th>In the concentrated antigen</th>
</tr>
</thead>
<tbody>
<tr>
<td>A 6.2ₕ</td>
<td>3.5</td>
<td>6.5</td>
<td>7.5</td>
</tr>
<tr>
<td>B 5.2</td>
<td>Tₖ</td>
<td>5.5</td>
<td>6.0</td>
</tr>
<tr>
<td>C 4.2</td>
<td>T</td>
<td>4.8</td>
<td>5.2</td>
</tr>
<tr>
<td>D 3.2</td>
<td>T</td>
<td>3.4</td>
<td>4.0</td>
</tr>
<tr>
<td>E 2.2</td>
<td>T</td>
<td>T</td>
<td>2.8</td>
</tr>
<tr>
<td>F 1.2</td>
<td>T</td>
<td>T</td>
<td>2.5</td>
</tr>
<tr>
<td>G 0.2</td>
<td>T</td>
<td>T</td>
<td>1.7</td>
</tr>
</tbody>
</table>

ₕInfective titer expressed in log 10.
ₖCell toxicity until dilution 10⁻¹.

Identical experiments were made with three uncontaminated vaccines. Eluted and concentrated antigen of these vaccines were negative in the three passages performed.

**DISCUSSION**

The classical methods of innocuity control are based on 100 observations by the inoculation of 0.1 ml, either in cattle tongue (6), cells (3) or suckling mice (7). When all observations are negative there is still a 4.5% chances that the vaccines are infectious with a possible error of 0.01 (3). The security of the method can be considerably augmented by increasing the number of observations or the inoculated volume (7). The most efficient way to increase the volume of inoculum is by elution of the antigen from the aluminum hydroxide and subsequent concentration (9).

The procedure described in this paper reduces 200 ml of antigen to a 4 ml inoculum that is one ml of eluted and concentrated antigen correspond to 50 ml of vaccine. If 3 Roux bottles
are inoculated with 1 ml per bottle, a total of 150 ml of vaccine are controlled instead of the 10 ml used in the conventional methods (3, 6 and 7).

The data in Table 1 show that the titer of the virus per ml of the eluted and concentrated antigen is on average 10 times higher than that of the contaminated vaccine. This fact combined with the increase of the inoculum volume makes this procedure at least ten times more sensitive than the conventional methods to detect infectious virus in aluminum-hydroxide inactivated FMD vaccines. On the other hand, the possibility of failing to detect active virus in a one ml sample of the eluted and concentrated antigen from an infectious vaccine is 0.03 with P = 99%. Moreover, the method has the advantage that the toxicity of the whole vaccine, of the supernatant or of the eluted antigen is eliminated. The elimination of toxicity is of great importance if the test has to be done with vaccines which contain saponin.

REFERENCES