Viability of Foot-and-Mouth Disease Virus in Oil Emulsions (34234)

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The adjuvant effect on antibody production of antigen emulsified in oil has been reviewed (1) and the mechanism of its action was analyzed (2). These authors conclude that the major action of the oil emulsion is immobilization of the antigen. Oil droplets containing antigen remain at the site of injection or are transported to distant sites in the lymphatic system where the antigen is slowly released. In addition to immobilization of antigen the capacity of the oil to stimulate protein synthesis in cells of the mononuclear series is common to other nonspecific stimulators of protein synthesis.

In investigations carried out with pigs, oil adjuvated foot-and-mouth disease virus (FMDV) vaccines were superior to vaccines prepared with aluminum hydroxide although the appearance of antibodies was delayed when oil adjuvants were used. These studies also emphasized the necessity of having an initial antigenic mass of sufficient quantity to initiate production of antibodies (3).

Results obtained in our laboratories from large scale investigations of FMDV vaccines emulsified in oil adjuvant showed less promise than results obtained under more stringently controlled conditions. Immunity to FMDV is best induced when the entire capsid of the virus is used as the antigen (4); therefore it is important to determine if the capsid is intact after emulsification of FMDV in oil and secondly to determine the amount of virus retained by the oil adjuvant as compared to that adsorbed by aluminum hydroxide.

Materials and Methods. Foot-and-mouth disease virus type O Vallée, subtype O_1 (Deodoro) adapted to young adult mice (5) was subsequently adapted to monolayers of BHK-21 clone 13 cells. Doses of 0.2 ml of the different preparations were injected subcutaneously into 5-week-old adult Swiss mice. Suckling mice are 100 times more sensitive to this virus than adult mice and were inoculated subcutaneously with 0.05 ml of each solution.

Aluminum hydroxide was obtained from the Brazilian Department of Agriculture and prepared according to the technique of Wilstatter. The mineral oil Drakeol 6VR (Pennsylvania Refining Co., Butler, Pa.) was sterilized by boiling and the emulsifier was Arlacel A (Atlas Powder Co., Wilmington, Delaware). The oil emulsions were prepared as described elsewhere (2). Serial $\log_{10}$ dilutions of virus were made in phosphate-buffered saline and further mixed with an equal volume of the adjuvants or phosphate-buffered saline. To ascertain the presence of infectious virus, serial $\log_{10}$ dilutions of virus without adjuvants were inoculated into suckling mice; 6–8 mice were used per dilution.

The following abbreviations were used in the text: OA is an oil emulsion obtained by mixing oil–Arlacel with an equal volume of aqueous phase; O signifies a mixture of oil and equal volume of aqueous phase; A signifies a mixture of Arlacel (10% v/v) with an equal volume of aqueous phase; DEAE-D is diethylaminoethyl-dextran (Pharmacia, Uppsala).

Results. The amount of infectious virus present in the aluminum hydroxide treated preparations (a), and in the oil emulsion (b) was determined by mouse inoculation. Table I shows the results of two experiments. The decrease in infectivity seen when the virus was emulsified with oil (99.9%) was greater than in the aluminum hydroxide preparations (54 to 85%)

Since it is known that the complete viral FMDV capsid is the best antigen available to produce the appearance of protecting anti-

TABLE I. Reduction in Titer of Serial Dilutions of FMD Virus Mixed with Aluminum Hydroxide and Oil-Arlacel.

	LD_{50}/ml in adult mice	
Control	$10^{5.30}$	$10^{5.0}$
Al $(OH)_3$	$10^{5.03}$	$10^{4.2}$
OA	$10^{2.30}$	$10^{2.2}$

bodies, this result shows that the oil emulsion makes immediately available to the inoculated animal only about 0.1% of the total mass of antigen inoculated, versus 15% to 46% for the vaccine prepared by aluminum hydroxide. This would be true however only if the retained antigen were not degraded during the emulsification.

In order to determine the viability of the immobilized virus, the (b) preparation was serially diluted in PBS containing 1 mg/ml of DEAE-dextran. As shown in Table II, the titer of the virus recovered after dilution in DEAE-dextran is almost equal to that of the controls. That indeed DEAE-dextran is able to break down the emulsion is shown in Table III where dilutions were made in PBS alone as well as in PBS containing the polymer: a full recovery is not obtained without the polymer. This polymer is however without effect on binding of the virus to aluminum hydroxide and on the infectivity of FMDV "O", injected subcutaneously in suckling mice, as shown in Table III.

Having thus established that an oil-emulsion immobilizes 100–400 times more virus in its infectious form than does $Al(OH)_3$ and that the emulsion can be broken down by serial dilution in PBS containing DEAE-dextran, it was next investigated if the direct addition of DEAE-dextran to an oil emulsion

TABLE II. FMD Virus Recovery from an Oil Emulsion by Dilution in PBS Containing 1 mg/ml DEAE-Dextran.

	LD_{50}/ml in adult mice	
Control	$10^{6.3}$	$10^{5.82}$
DEAE-dextran	$10^{6.45}$	$10^{5.85}$
OA	$10^{3.3}$	$10^{2.95}$
diluted in DEAE-D	$10^{6.1}$	$10^{5.82}$

would break down the emulsion without impairing the immobilizing capacity of this emulsion. It was not possible to obtain emulsions with a concentration of DEAE-dextran as low as 31 μg without a mixer. Final concentrations of 200, 400, and 800 μg of DEAE-dextran were added to serial dilutions of virus subsequently mixed with oil and Arlacel. No emulsions were obtained, but there was a $\log_{10}$ reduction in infectious titer with such preparations, as shown in Table IV.

TABLE III. Comparison of the Effect of DEAE-Dextran (1 mg/ml) on FMDV Infectivity in Suckling Mice of an Oil Emulsion and $Al(OH)_3$.

	LD_{50}/ml in suckling mice	
PBS	$10^{6.90}$	$10^{7.9}$
OA diluted in PBS	$10^{5.3}$	
DEAE-D	$10^{6.73}$	
DEAE-dextran		$10^{7.8}$
$Al(OH)_3$[a]		$10^{6.3}$
plus DEAE-D		$10^{6.53}$
diluted in PBS[b]		$10^{7.90}$

[a] Virus diluted serially in PBS and $Al(OH)_3$ added in equal volume to all dilutions.

[b] Virus–$Al(OH)_3$ mixture serially diluted in PBS.

Finally, the ability of oil without Arlacel and Arlacel without oil to reduce the infectious titer of a viral preparation was tested in presence of DEAE-dextran. Comparisons were made in this respect with a true oil emulsion. Table V shows that Arlacel had no

TABLE IV. Effect of Various Concentrations of DEAE-Dextran on the Infectivity of FMD Virus Mixed with OA.

Conc (μg)	LD_{50}/ml in adult mice
Control	$10^{8.05}$
OA	$10^{5.8}$
+ DEAE-D, 200	$10^{7.2}$
400	$10^{7.0}$
800	$10^{7.2}$

influence on the virus titer, that oil alone mixed with DEAE-dextran also had no effect, and that a reduction in titer is seen in the presence of DEAE-dextran only when oil and

TABLE V. Effect of Two Different Concentrations of DEAE-Dextran on FMD Virus in Oil, Arlacel, and Oil Plus Arlacel.

	Conc (μg)	LD_{50}/ml in adult mice
OA		$10^{2.36}$
+ DEAE-D,	250	$10^{3.7}$
	1000	$10^{5.36}$
O + DEAE-D,	250	$10^{6.0}$
	1000	$10^{6.0}$
A + DEAE-D,	1000	$10^{5.7}$

Arlacel are used jointly. However, this reduction in titer is less pronounced than the one observed in the experiments in which oil and Arlacel were used alone. The higher the content in DEAE-D, the lesser was the reduction observed. Arlacel alone or oil alone added to a viral preparation likewise had no influence on the virus titer.

Discussion. Although oil adjuvants have shown great promise for immunization programs, there has been limited application of this adjuvant in FMDV vaccination. This is particularly true in South America, where the use of oil emulsified FMDV vaccine is being considered. Three major factors must be considered prior to the widespread use of oil adjuvants. (i) The preparation of emulsions on a commercial scale, (ii) the inoculation of these viscous preparations without provoking strong fibrotic reactions at the site of inoculation, (iii) evaluation of the optimum mass of antigen to be inoculated. The protein mass needed to reach a threshold of reaction in the vaccinated animals must be reconsidered when a new adjuvant is utilized.

Our experiments show that the oil emulsion has no detrimental effect on the viability of FMDV. It is thus presumed that the viral proteins are not destroyed during the emulsification process. The amount of protein, taking the infectious virus as reference, immediately available for antibody formation is much lower when an oil adjuvant is used than when aluminum hydroxide is used, 140 to 400 times more antigen is immobilized in the emulsion in the latter case. This indicates that a careful evaluation of the protein mass required to initiate antibody formation is needed when oil emulsions are used, otherwise the vaccine will be ineffective.

These experiments establish that the oil emulsion is needed in order to achieve a maximal immobilization of the virus. Only a small percentage of the virus is immobilized when DEAE-D is added to the oil–Arlacel mixture in such a manner as to prevent formation of a true emulsion. Since it is not known at what rate and in what fashion this remaining virus will be released into the animal tissues, such preparations must be evaluated antigenically in comparison with aluminum hydroxide vaccines. In addition the oil–Arlacel and DEAE-dextran combination is easier to inject than the oil emulsion.

Summary. The FMD virions are not destroyed during emulsification with mineral oil and Arlacel. The oil emulsion absorbs 140 to 400 times more virus than does aluminum hydroxide. The oil emulsion can be broken down by dilution in PBS containing 200 μg/ml DEAE-dextran.

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