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STUDIES ON THE PRODUCTION OF SPECIFIC HYPERIMMUNE ANTISERA AGAINST TYPE A F.M.D. VIRUS

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ABSTRACT. **Production of hyperimmune antisera against type A FMD virus was performed by two different methods and antigens using guinea pig and BHK_{21} cell line.**

In the first experiment antigen was prepared on BHK_{21} cell line and then concentrated and mixed up with freund's complete akjuvant and inactivated Mycobacterium tuberclosis as stimulating factor.

In the second experiment hyperimmune antisera against type A of FMD virus was prepared by infection of virus to the foot pad of guinea pig and for obtaining better immune response inoculated FMD virus was first mixedup with saponine and live strain of Brucella S_{19}.

Key words: IMMUNE SERUM/ANTIGENS/VIRUSES/GUINEA PIGS/

INTRODUCTION

Production of specific hyperimmune antisera is aimed at detecting different types and subtypes of FMD and is also useful for determining the level of antigens

in related vaccine. FMD vaccine can be sufficiently immunogenic when it cours all prevalent new types and subtypes of the virus present in a region.

Besides the titre of antigens used in vaccine and serological tests which detect the level of present antigen in vaccine is very important. Therefore, possessing a potent hyperimmune antisera against each subtype of FMD virus, plays a fundemental role in producing a reasonable immune response among inoculated cattle.

MATERIALS AND METHODS

Antiser: (I),(II),(III)

- Preparation of concentrated FMD virus type A 1987 (Mardabad-strain)

a- FMD virus type A 1987 (Mardabad strain) was inoculated into BHK_{21} cell line (I) and above mentioned virus strain was also cultured in Bovine Epithelium of Tongue (Franckel) (II) and after appearing 100% C.P.E. the virus was collected and centrifuged for 30 minuts at 10000 RPM in order to delete the none specific proteins and cell debris.

b- Sedimentation of viral particles by the help of 7.5% Polyethyline glycol for 4 hours at 4°C and collection of sediments and resuspending it in P.B.S. PH=7.6 supplemented with inactivated complement at 1% dilution so the antigen is concentrated 25 folds.

c- The concentrated viral antigen is mixed up with equal volume of complete Freund's adjuvant and 5% tween 80 abd the homogeneous antigen is thus produced.

d- The viral Homogeneous antigen was inoculated in 0.1ml

volume at different dilutions to the Foot pad of guinea pigs. 28 days past inoculation 0.4ml of the antigen was inoculated subcataneously as booster. Finally, 7 days after booster,guinea pigs were bled and hyper - immune sera was separated. (1,2)

- Adaptation of FMD virus strain A 1987 (Mardabad)to the Foot pat of guinea pig. (III).

a- Adaptation of FMD virus was achieved after at least 5-6 times of serial passage (Invivo).

b- The adapted FMD virus was collected and inoculated into the foot pad of some other guinea pigs and harvesting the fluid of vesicules on Foot pad of guinea pigs one day later and leaving the animals to be recovered for one mounth.

c- Inoculation of adapted FMD virus combined with live Brucella S_{19} strain and saponine as a stimulating factor for better immune response, to the Foot pad of previously recovered guinea pigs by successive subcutaneus, intramuscular and intraperitoneal inoculations and finally bleeding guinea pigs and collecing hyper-immune sera two days after the last inoculation.(3).

Antigens (I) (II)

The antigens for serological study have been prepared according to the following methods.

(I) On mono layer culture of BHK_{21} cell line.

(II) On bovine epithelium of Tongue (Franckel)(4)

RESULTS & DISCUSSION

The result of comparative serological evaluations was obtained by 100% complement fixation test according

to Kolmer's method and reflected in tables 1,2,3, with respect to antisera produced in different ways.

In spite of the fact that non specific proteins and cell debris have been deleted in experiment No. by high speed centrifugation, but the results of comparative serological tests shown that the most specific hyperimmune antisera can be produced by adaptation of FMD virus to the foot pad of guinea pig, because in this method, non specific proteins in the viral antigen can not interefere in hyperimmune antiser production.

For this reason obtaining and having a potent hyperimmune antisera against each types and subtypes of FMD virus is very valuable for detecting new subtypes in outbreacks as well as determination of viral infectivity in produced vaccines. (5,6,7,8).

Table No.1

Serum diluation(I) / Antigen	1/10 1/20 1/30 1/40 1/50 1/60 1/70 1/80 1/90 1/100 1/110	T.s	T.Ag	T.ć	T.CH
(I)	4 ——→	0	0	0	4
(II)	4 4 2.5 Tr↓	0	0	0	4

Table No.2

Serum diluation (II) / Antigens	1/10 1/20 1/30 1/40 1/50 1/60 1/70 1/80 1/90 1/100 1/110	T.S	T.Ag	T.ć	T.CH
(II)	4 ——→	0	0	0	4
(I)	4 4 3 0.5 ↓	0	0	0	4

Table No.3

Serum diluation (III) / Antigens	1/10 1/20 1/30 1/40 1/50 1/60 1/70 1/80 1/90 1/100 1/110	T.S	T.Ag	T.ć	T.CH
(I)	4 ——→	0	0	0	4
(II)	4 ——→	0	0	0	4

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