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PRODUCTION OF FOWLPOX VACCINE VIRUS IN CHICK EMBRYO DERMAL TISSUE CULTUE

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SUMMARY

Chick embryo dermis (CED) cell cultures were prepared from skinpeeled off from 12 day old specific pathogen free (SPF) embryo. The cultures were grown in 199 medium containing 5 % calf serum and 10 % trypose phosphate broth (TPB). For primary cultures, suspensions containing 8 x 10^5 cells per ml were seeded in Roux bottles, culture tubes and 2 oz plastic bottles. Cell culture passaged 3-5 times without muh difficulty.

Canadian vaccine strain of lowlpox virus was propagated satisfactorily in CED subcultures. Cytopathic effect (CPE) was characterized by rounding of cells, ballooning of a few infected cells and frmation of intracytoplasmic inclusion bodies. The virus titers were in the order of $10^{7.6}$ TCD 50 per 0.1 ml.

Three experimental batches of cell culture adopted virus were prepared to be tested for immunization of susceptible birds. These batches were titrated in CED cultures, embryonated SPF eggs and susceptible chickens. The titers were higher on CED cultures. Four weeks old chickens vaccinated with approximately 10^5 TCD 50 developed good takes and remained apparantly healthy

during the period of observations. Protection was demonstrated against challenge with virulent fowlpox virus. Advantages of production of fowlpox vaccine in CED cultures instead of embryonated eggs were outlined briefly.

INTRODUCTION

Various investigators have reported preparation of cell culture fowlpox vaccine inn chick embryo cultures and effectiveness of such vaccines in their respective countries (1-7). Nevertheless, because of low virus titers obtained in infected cell cultures, the efficiency of these vaccines in endemic areas is yet to be proven. In 1974 El Zien et al. (8) reported development of chicken embryo dermis (CED) cultures which yield relatively a high virus titers and were found suitable for preparation of fowlpox vaccine (9,10).

In view of the obvious advantages of tissue culture origin vaccines the purpose of this report is to describe the production of fowlpox vaccine in CED culture and determine its efficiency to combat fowlpox as well as economically important disease to rapidly expanding poultry industry in Iraq under local conditions.

MATERIALS AND METHODS

Chick embryo dermis cultures : Primary CED were prepared by scraping genetally the skin of 12-day old embryos. After washing 3 times with Hanks balanced salt solution (HBS) they were digested several times with 0.05 % trypsin solution for 5-10 minutes at room temperature. The first two trypsinization were discarded, and the

following ones were collected in a bottle containing 5 ml of calf serum and stored in an ice bath. The dispersed cells after centrifugation at 1000 r.p.m for 5 minutes were resuspended in medium 199 supplemented with 5 % calf serum 10 % tryptose phosphate broth with 100 IU penicillin and 100 µg streptomycin/ml. The final cell suspension contained 8 x 10^5 cells / ml; 5 ml and 25 ml of cell suspensions were seeded in 2 and 25 oz. plastic bottles. Monolayers were obtained 84-72 hours later.

Subcultures were prepared by treating the monolayers with 0.25 % trypsin. Two bottles were prepared from one resulting in a final suspension of approximately 2 x 10^5 cells per ml. The cultures were incubated at 37 °C in a stationary position.

Virus strains : Canadian strain of fowlpox virus was kindly supplied by Dr. Al Zein of Fanaer Regional Pultry Laboratory in Lebanon. The strain has been used since 1970 in the Phylaxia Institute Budapest Hungary for the production of fowlpox vaccine by the conventional methods of virus propagation on chorioallantoic membrane (CAM) of embryonated eggs. The strain had undergone 5 passages in CED cell cultures at Fanaer Laboratory. For preparation of stock virus a vial was reconstituted in 1 ml of sterile nutrient broth and 0.2 ml of 1/10 dilution of the virus was inoculated into each of four CED monolayers grown in 2 oz. bottles. The infected fluid was harvested 6 days after infection when cytopathic effect was observed in approximately 80 % of the monolayers, and was stored at -20 °C after dispensing in vials.

Ghraib fowlpox (AFP) isolate. AFP isolated from an outbreak of fowlpox among 16 week old birds at Veterinary Research Institute, (now known as Kindy company) Abu-Ghraib, was used in 1st and 2nd passage level on CMA for challenge.

Preparation of cell culture FP vaccine : one ml of the 4th passage of Canadian FP virus in CED cultures in our laboratory was inoculated into each of the 16 oz. plastic bottle with 3rd passage level of monlayers of CED cultures. After 1 hour adsorption of the virus at 37 °C, 25 ml of 199 medium containing 3 % calf serum and usual concentration of TPB and antibiotics were added to each of the bottles. Following inoculation, infected cultures were incubated at 37 °C and were examined for CPE for eight days. When CPE was observed in about 80 % of the monolayer, the cultures were frozen at -20 °C and thowed twice. The infected fluid was centrifuged at 1000 r.p.m for 5 minutes and supernatant was used as vaccine after diluting it with equal volume of glycerine buffered saline.

Vaccine titration : Titration of the fowlpox cell culture vaccine was done by inoculating 2 CED cell culture 2-oz. bottles eachone with 0.2 ml of respective dilutions of vaccine. The virus was adsorbed for one hour at 37 °C before adding medium. The final reading for CPE was taken on the 8th day following inoculation and titers were expressed as TCD 50 / ml according to Karber method.

Titration on the CAM was done by inoculating 0.1 ml of the respective virus dilutions via artificial air sac on

hours were discounted. All the living eggs were opened on the 5th day after inoculation and pock forming unit (PFU) was estimated by the presence of pock lesions

Four-week old leghorns chickens were also used for vaccine titration. Five chickens were used for each of the virus dilution and each chicken was inoculated by feather follicular (FF) and wing-web (WW) methods. Takes were recorded for 15 post inoculation day and titers were expressed as chick infected dose (CID/50/ml).

Potency test : Each of the three dilutions viz undiluted, 1/5 and 1/50 were incubated into five 4-week old leghorn chickens by FF method with contact controls. Local reactions were recorded at 18 days after vaccination. Vaccinated along with control group were challenged with virulent strain of fowlpox virus (AFV) by the FF method and the birds were observed for local and general reactions for 21 days post challenge (DPC) (Figure 1).

RESULTS

Cytopathology of FP vaccine in CED cultures : The CPE characterized by rounding of cells and ballooning of a few infected cells. This was first noticed 3 days post-infection with approximately to 10^6 TCD 50 per culture bottle. CPE progressed to involve almost the whole monolayer 2-3 days later. Both the fibroblastic (prodominant type) and epithelials appeared to be involved. FP virus infected cells when stained with Giemsa stain

showed intracytoplasmic inclusion and during the later stages most of the infected cells showed granular cytoplasm and vacuoles. Titer of the cell-free virus on the 4th passage of CED cultures when CPE was almost complete was in the order of $10^{7.6}$ TCD per 0.1 ml.

Titration of FP vaccine on CAM, in CED cell cultures and in chicken : Table 1 summarizes the mean titers of the FP cell cultures vaccine in various systems. Titers when expressed as TCD 50 in CED cultures were higher than the ones obtained on CAM (PFU 50) and 50 % - chicken - infective dose (CID 50).

Safety and potency test : When 4 - week old chciken were inoculated by FF and wing web (W/W) methods with undiluted infected tissue culture fluid each chicken receiving approximately 10^7 TCD 50 no unwanted reaction was observed in the vaccinated chicken although takes were pronounced in all the chickens. Vaccination had little influence on the general condition of the birds.

Abu-Ghraib fowlpox isolate strain used for potency test gave severe local reaction when applied by FF method and caused tensive general reaction when given I/V to susceptible 6 week old chickens. Results of potency test summarized in Table 2 indicate that 1:50 dilution of vaccine (10^5 TCD 50) gave solid protection in 4 out of 5 vaccinated birds. One showed abortive post-challenge reaction indicating that it might have been partially immunized. Ten out of ten birds vaccinated with 1 : 5 dilution (10^6 TCD 50) to be used as vaccinal dose provide solidly immune to challenge with local virulent strain of fowlpox virus which gave several local reaction in contact controls. Observation

following challenge were made for 21 days period. Although general conditions of the control birds were severely affected during the 2nd and 3rd weeks after challenge, none of them showed generalized lesions during the period of observation.

DISCUSSION

Selective ability of fowlpox virus to produce pathological lesions in skin tissues of infected chickens prompted the study of growth of fowlpox attenuated strain in these CED cell cultures. Furthermore, as the CED cells are easy to grow and can be maintained for 2-3 weeks and yielded high titer of the virus seem the system of choice for in vitro growth of FPV, and development of tissue culture vaccine against fowlpox. In addition to the fact that CED culture is less expensive than the chick embryo (CE) host system, the vaccine produced has the well documented advantages of a tissue culture origin vaccine, namely easy to standardize and produce homogenity, purly, and strict fungal and bacterial sterelity in the final product. As CED cell culture yielded higher titer of fowlpox vaccine than those of other proposed FPV tissue culture origin vaccine, allows preparation of more potent vaccine with a high virus contents per field dose. Titer reported by various investigators so far in chick-embryo and duck-embryo culture was about 10^5-$10^{6.2}$ TC 50 per ml (1,6,7,10) which are close to the minimum quantity of virus required to successfully immunize chickens (11), but under our experimental conditions CED culture system gave a titer of

$10^{8.6}$ / ml. Our observations reported here are in agreement to the ones reported earlier by El Zien et al. (8) and suggest that CED cell culture vaccine may proved to the vaccine of choice to immunize poultry populations.

Table 1: Titers of cell culture fowl pox attenuated starin in various system

systems	Log titer/ml
CED cell culture	8.2 TCD_{50}
CAM	8.1 PFU
Chicken wing-web	4.1 CID_{50}
Chicken follicle	4.4 CID_{50}

CED = Chicken embryio dermal cells.

Table 2: Potency test of cell culture FP vaccine

Vaccine dilation	No of chicken vaccinated	No with 'take'	Reaction challange
Undilated	5	5	Nil
1/5	10	10	Nil
1/50	5	4	1 reacted but reaction subsided in 14 day
Contact control (5)	-	-	Sever local reaction in all

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تحضير لقاح جدري الدجاج على خلايا الزرع النسيجي المحضرة من ادمة اجنة الدجاج

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الخلاصة

تم تحضير خلايا الزرع النسيجي من ادمة اجنة الدجاج الخالي من المسببات المرضية بعمر 12 يوم وقد نميت هذه الخلايا في الوسط الزرعي الحاوي على 5٪ مليلتر من مصل العجل و 10٪ من TPB وكان تركيز الخلايا الاولية المزروعة 8×10^5 خلية لكل واحد مليلتر.

استخدمت هذه الخلايا لتنمية العترة اللقاحية لجدري الدجاج Canadian vaccine strain وكان تاثير الفيروس على الخلايا واضح وتميز بتكوين خلايا مكورة مع وجود الجسم الضمين Inclusion bodies داخل سايتوبلازم اخلية. وقد تم تحديد عيارية الفيروس المنتج وكانت $10^{7.6}$ لكل 0.1 مليلتر.

كما تم معايرة الفيروس اللقاحي على الزرع النسيجي لاجنة الدجاج وعلى الغشاء اللقائقي المشيمي لاجنة الدجاج وكذلك على دجاج وكذلك على دجاج بعمر اربعة اسابيع وكانت الجرعة اللقاحية الحاوية على 10^5 فيروس لكل مليليتر كافية لاعطاء حصانة جيدة للدجاج الملقح عند التعرض الى الفيروس الضاري لجدري الدجاج.