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Preparation and Evaluation of a Live Modified Fowl Pox Vaccine Using Chicken Embryo Fibroblast Cell Culture

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Summary

Preparation of a safe and immunogenic vaccine against fowl pox disease using chicken embryo fibroblast cell (CEF) with an egg adapted fowl pox virus is described. The prepared vaccine, administred by wing web stab method, has induced complete protection against experimental fowl pox challenge virus. For the final evaluation of the efficacy of the prepared vaccine the authors propose this vaccine to be used in the field urder the supervision of veterinary clinicians.

Introduction

A few decades ago fowl pox was known as a disease with considerable economic importance to the poultry industry, but it was effectively controlled by routine vaccination using different type of vaccines. In recent years, intensive method of mangement may have played a role in making fowl pox a relatively uncommon disease. Nevertheless, foci of infection have persisted in some areas of the country so that small-scale outbreaks appear in those areas almost regularly. For this reason, vaccination of birds against fowl pox is still carried out constantly. Fowl pox virus has been propagated successfully in various cell culture of bird origin since 1928 by several investigators (Bierbaum and Gaede,. 1935; Findley, 1928; Loewenthal, 1928). The modern technique for the propagation of fowl pox virus in cell culture was first used by Kohler and Schwobel (1956). Benegelodroff and Schnieider (1963) demonstrated that vaccines prepared from fowl pox and pigeon pox viruses, propagated in chick embryo cell culture, could produce desired immunisation against fowl pox disease in birds. Gelenczei and Lasher (1968) propagated fowl pox, pigeon pox and turkey pox viruses in duck and chicken embryos and the growth and

antigenicity of each of them was comparatively tested. In the present study, fowl pox virus previously propagated in chick embryo was adapted to chick embryo fibroblast cell culture and an immunogenic vaccine was prepared This vaccine was proved to be as effective as the vaccine now being produced on chorioallntoic membrane in embryonated eggs.

Materials and methods

Virus strain: The virus strain used in this work, was a modified strain of fowl pox virus currently used for preparation of vaccine at this Institute It produces a titre of 10^6 EID_{50}/ml..

Preparation of cell culture:All embryonated eggs used for preparations of chick embryo fibroblast (CEF) were specific pathogen free (SPF) and were obtained from a commercial breeding flock (Lohmann, Germany). Primary and secondry CEF cultures were prepared in Roux bottles as described by Baxendale (1971) using EMEM and Hanks media containing 7-10 % fetal calf serum and supplemented with triptose phasphate broth.

Titration:Titrations were carried out with ten fold dilutions of experimentally prepared vaccine in Hanks balanced salt solution without serum and 0.1 ml of each dilutions were inoculated onto the previously prepared chicken embryo fibroblast subcultures. The cells were checked daily for the appearance of cytopathic effect and the end point ($TCID_{50}$/ml) were calculated by the Reed and Munch (19380) method.

Vaccine preparation: Two batches of vaccine were prepared from egg adapted fowl pox virus grown on CEF. Batch A and B were prepared from the virus at 12th and 13th passage, respectively. Vaccines were freeze-dried using the conventional method for freeze-drying of poultry vaccines.

Safety and potency: The freeze-dried vaccines were reconstituted by saline to contain 10^3 $TCID_{50}$/ml as one vaccinal dose. Vaccination was carried out by wing web stab method delivering 0.01ml to each chicken. Three groups of 5 weeks old SPF chickens were used as follows:

Group 1- 10 chickens each recieved fowl pox vaccine prepared by the seed virus at 12th passage. *Group 2*- 10 chickens each received fowl pox vaccine prepared by the seed virus at 13th passage. *Group 3*: 8 susceptible chickens were kept unvaccinated, as controls, in the same battery throughout the experiment. The inoculaton sites of all chickens were examined daily for the evidence of **Take** of the vaccine or eventual untoward reactions for a period of 3 weeks. All chickens, including controls, were challenged by scarification of one drop, 0.005 ml, of virulent virus with the titer of $10^{6.2}$ EID_{50}/ml on their combs.

Results

The cytopathic effects (CPE) of the egg adapted fowl pox virus infected cell culture, as rounding of the cells followed by the second phase of degeneration, were observed.The titer of the virus increased and reached a maximum of $10^{6.5}$ $TCID_{50}$/ml on the 14th passage. The CEF cells showed complete destruction and sloughed off the glass surface 3-4 days after inoculation of the virus.

All 20 vaccinated birds showed a mild reaction at the inocculation site indicating vaccine reaction (Take). Three weeks after vaccination all 28 birds including 8 unvaccinated birds (control) were challenged with a field strain of virulent virus. All vaccinated birds resisted against virulent virus, while 8 control birds showed a severe reaction at the inoculation site.

Discussion

Avian pox virus have been propagated in various cell culture system for decades as early as 1928 . Several investigators (Bierbaum and Gaede,. 1935; Findley, 1928; Loewenthal, 1928) propagated fowl pox in chicken embryo tissue extract. The modern trypsinisation cell culture technique for the propagation of fowl pox was first applied by Kohler and Schwobel (1956). A few years later Mayer and Kalcher (1960) reported the propagation of pox virus in chicken embryo fibroblast cells. Bengelsdroff and Schneaider (1963) demostrated that vaccines prepared from fowl pox viruses propagated in chick embryo cell culture were suitable for practical use. A simillar finding was reported by Bamberger and Markovits (1965). In the present study fowl pox virus was propagated in chicken fibroblast cell culture. Primary monolayer fibroblast cell culture were derived from 10 day old chicken embryo. The vaccine virus was given serial blind passages for adaptation to cell culture. Cytopathic changes were evident after 3-5 days. These changes consisted of rounding/aggregation of cells with cytoplasmic strands and formation of intracytoplasmic vacuoles. The results of the experiment revealed that fowl pox virus previously propagated on chicken embryo can be easily adapted to chicken embryo fibroblast cell culture and the vaccine prepared on these cells has no significant difference in titer from the vaccine prepared in chicken embryo. Although the effect of virus concentration in vaccine had already been tested by Winterfield and Hitchner(1965) and they had found that 10^4 EID_{50}/ml was the minimum titre to protect chicken against a virulent fowl pox virus, in our experiment the minimum titre of fowl virus needed for protection was found to be 10^3 $TCID_{50}$/ml. The vaccine was tested for safety and potency in chickens of 5 weeks age by wing web stab method. Vaccinated birds were observed for 3 weeks and take reaction were checked at the end of this period. We

conclude that the immunogenic efficacy of the vaccine prepared in the chicken embryo fibroblast is as good as that of the one prepared in chick embryo.

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