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DEVELOPMENT OF AN ATTENUATED LIVE VIRUS VACCINE AGAINST SHEEP POX *

By :

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Introduction

In a previous paper (2) we reported the possibility of producing *sheep pox virus (SPV)* on a large scale in tissue culture.

Production of tissue-culture virus is easy and cheap. Its titre is higher than the virus obtained by the Borrel Pustule technique. Furthermore, it is free of contamination, which may cause severe problems in the control of the disease.

Tissue culture virus from early passages, when injected into susceptible animals, causes high fever and large local reactions, with generalization in some cases.

After 30 consecutive passages in sheep kidney (SK) cells the virus was sufficiently attenuated to induce solid immunity in sheep without causing severe reactions.

Materials and Methods

Virus vaccine: The strain designated by us as RM/65 originated from a natural outbreak of sheep pox which occurred some years ago in Yugoslavia and was kept at the Institute for Microbiology and Infectious Diseases of Animals, University of Munich, Germany until used.

The attenuation of virus was carried out by one of us (H. Ramyar) while working on a FAO fellowship in the above Institute.

The virus was first injected intradermally into a sheep. The animal reacted strongly to the injection and large pustules appeared on both sides of the thoracic region. The virus was harvested aseptically, ground with an Ultra-Turrax Type TP 18/2 (Janke und Kunkel K.G., Fabrik Chem. Phys. Apparate und Maschinen, Staufen i. Br., Germany), and SK primary cell cultures were infected with the ground material diluted 1/10 in VM 3 (3).

After 2 serial passages cytopathogenic effects (CPE) were observed as early as 48 hours post-infection.

The virus was passaged 80 times.

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Preparation of Vaccine

For the evaluation of the safety and antigenicity of culture virus large amounts of the 30th, 40th and 50th passage levels were produced in SK cells cultured in Blake bottles: 96 hours post-infection, bottles were transferred to a — 30° C. deep freeze. Frozen material was thawed at room temperature, passed through one layer of gauze, distributed in 2 ml. amounts in 5 ml. vials and lyophilized in a Stokes freeze-drying machine, model 902-005-9 (F. J. Stokes Machine Company. Philadelphia, 20-Pa. USA) at —40° C. under 50 micron vacuum within 24 hours.

The virus was titrated before and after lyophilization using susceptible sheep and SK cell cultures. Results were calculated according to Kärber (1). No marked drop in titer was observed.

Keeping Quality of Lyophilized Vaccine

The viability and conservation of the immunizing power of the virus are very good. According to our results, the antigenicity of the vaccine remains intact even after storage for more than 2 months at room temperature (varying from 21° C to 26° C) and 20 days at 37° C.

Safety and Potency Tests

The contents of each vial were reconstituted in 200 ml. of sterile distilled water: 335 sheep in the laboratory and over 11,000 sheep in the field were vaccinated by the subcutaneous route with 1 ml. of the diluted live vaccine. None of the vaccinated animals showed large local reactions and there was no single case of abortion in pregnant ewes.

On one occasion, the vaccine was administered to a flock already infected with sheep pox. The disease ceased following vaccination and after 4 days there was no report of new infection.

Ninety-five out of 335 sheep immunized in the laboratory together with 12 controls were challenged 14 days post-vaccination with a virulent virus.

Vaccinated animals each received 0.5 ml. intradermally of undiluted Roumanian strain challenge virus. Controls were injected by the same route and with the same amount of challenge virus but diluted 1/200.

All vaccinated sheep resisted the challenge, while the controls showed high fever and a typical pox reaction at the site of inoculation.

The duration of immunity established by this vaccine is now under study and will be reported later.

Discussion

The live attenuated sheep pox virus vaccine can be utilized with good results in countries where sheep pox outbreaks occur with high mortality and great economic loss.

The preparation of the vaccine, when compared with the old type of tissue or lymph vaccines, is simpler. The vaccine is not bulky Its keeping qualities at

unfavourable temperatures make its use possible in places lacking cooling facilities.

SPV grows in a wide variety of mammalian cell cultures not naturally susceptible to this agent. In experiments with heterologous cells, we were able to adapt and cultivate the virus in monolayers prepared from pig kidney, pig testis, calf kidney, calf testis, kid kidney, kid testis, chicken fibroblasts and the BHK-21 stable cell line.

Summary

Laboratory experiments and field investigations showed that the live modified sheep pox virus vaccine, strain RM/65, is completely innocuous after 30 consecutive passages in sheep kidney cells.

Sheep vaccinated with a single dose of vaccine resist the challenge dose of virulent virus.

The vaccine does not lose its immunizing power when kept for more than 2 months at room temperature and 20 days at 37° C.

Vaccinated animals do not spread the disease.

The attenuated virus is capable of stopping outbreaks in the field. Since the immunity is established within a short time after vaccination, the vaccination of apparently healthy animals in infected flocks prevents progress of an outbreak.

Zusammenfassung

Entwicklung einer abgeschwächten Lebendvirus-Vaccine gegen Schafpocken

Laboratoriums- und Feldversuche zeigten, dass die aus dem Schafpocken-Virus, Stamm RM/65, hergestellte modifizierte Lebendvakzine nach 30 aufeinanderfolgenden Passagen auf Schafnierenzellen völlig unschädlich ist. Schafe, die mit nur einer einzigen Vakzinedosis behandelt wurden, widerstehen einer Belastung mit virulentem Virus.

Auch nach mehr als zweimonatiger Aufbewahrung bei Zimmertemperatur und nach 20 Tagen bei 37° C verliert die Vakzine ihre Immunisierungskraft nicht. Vakzinierte Tiere verbreiten die Erkrankung nicht. Das abgeschwächte Virus ist imstande, in der Praxis Ausbrüche aufzuhalten. Da sich die Immunität innerhalb kurzer Zeit nach der Vakzinierung ausbildet, kann diese bei offensichtlich noch gesunden Tieren in infizierten Herden einem weiteren Umsichgreifen der Erkrankung vorbeugen.

Résumé

Le développement d'un virus vaccin atténué contre la clavelée

Des expériences de laboratoire ainsi que des éssais sur le terrain, prouvent que le virus vaccin modifié, souche RM/65, est complètement inoffensif après 30 passages successifs sur les cellules rénales de mouton.

Les moutons vaccinés avec une seule dose de ce vaccin résistent à l'épreuve à l'aide d'un virus virulent.

Le vaccin ne perd pas son pouvoir antigénique après un stockage de 20 jours

à 37° C ou de plus de 2 mois à la température du laboratoire.

Les animaux vaccinés ne diffusent pas la maladie.

Le virus atténué est capable de bloquer sur le terrain la propagation d'une infection naturelle. Comme l'immunité s'établit rapidement, la vaccination des moutons apparament sains dans un troupeau infecté permet l'éradication de l'épizootie.

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