Isolation of Paravaccinia Virus from Sheep

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Viruses belonging to paravaccinia subgroup cause many diseases such as bovine papular stomatitis, Orf, milker's nodules, chamois contagious echthyma and others [2]. In Japan, Kumagai et al. [1] recently isolated paravaccinia virus from two calves suffering from bovine papular stomatitis. By sero-epidemiological survey, they found the widespread occurrence of this disease in cattle population in the northern part of main island of Japan. Furthermore, the paravaccinia virus was also isolated from Japanese antelope (cited in Animal Hygiene Weekly, No. 1435, Jan. 31, 1977) and sheep in the same area (Dr. M. Shimizu, personal communication). In the central part of Hokkaido, a pox-like disease of sheep, which was characterized by the formation of papules and vesicles on the skin of the lips and sometimes around the nostrils and eyes, has been recognized since 1965 (Dr. K. Kagota, personal communication). The clinical symptoms characteristic of contagious echthyma in sheep (which is caused by ovine Orf virus) were also observed by Dr. Y. Kawakami, who detected paravaccinia virus particles in thin sections of scabs from the clinically diseased sheep and successfully transmitted this disease to normal sheep by inoculating the scab materials, but no virus isolation was made (personal communication).

Tumor tissues from olfactory mucosa from 12 sheep were brought in our laboratory. Several tissues including the tumors from these sheep were examined histopathologically or electron microscopically, and the disease was diagnosed as papillary adenoma and adenocarcinoma in sheep [7]. During investigation of the etiologic agent of the disease, a kind of virus particles was found in thin sections of all tumor tissues examined and it resembled morphologically to visna virus [7]. When some of these tumor tissues were directly cultured or co-cultivated with normal sheep choroid...
plexus or testis cells, we found the visna-like virus in 3 out of 4 cell cultures. Only one cell culture showed cytopathic effects and it also contained ovine Orf virus. The present short communication describes the isolation of Orf virus from these tumor cells. However, the Orf virus appeared to be not responsible for the disease, because no virus particle was detected in the tumor tissues and no pox lesion was observed in sheep, from which the tumor tissues were obtained.

Details of the tissue culture technique were described previously [4]. Briefly, portions of neoplastic tissues from sheep with papillary adenocarcinoma or normal tissues were collected in culture medium. The tissue specimens were minced in small sterile flasks to fragments smaller than 1 mm³, and transferred to sterile culture flasks. Three to 4 days after planting, the cultures were shaken gently to dislodge the larger tissue fragments from the growth surface, and medium and tissue fragments were discarded and replaced with fresh medium. In serial passage, the monolayer cells were dispersed with 0.02% ethylenediaminetetraacetic acid (EDTA) in calcium and magnesium-free phosphate-buffered saline and transferred to one or two new culture flasks. For co-cultivation with normal sheep cells, minced tumor tissues were planted in culture flasks containing previously (12–24 hr) seeded but nonconfluent monolayers of choroid plexus or testis cell cultures.

For electron microscopical examination, thin sections from the cell sheet with a cytopathic effect were prepared as described previously [5]. The culture fluid from these infected cultures was centrifuged at 26,000 rpm for 90 minutes and the pellet was resuspended in a small amount of phosphate-buffered saline (PBS, pH 7.2). This virus preparation was purified by centrifugation in 15–60% (wt/vol) sucrose gradient prepared in PBS with Hitachi SW 65 rotor at 40,000 rpm for 90 minutes. Samples from each fraction were stained with 2% sodium silicotungstate (pH 7.2) for electron microscopic examination [5]. The virus particles characteristic of the paravaccinia virus were found in both preparations. In the thin sections, many ovoid particles measured 260×160×135 nm in size were present in cytoplasm of the infected cells (Fig. 1). Particles with the same appearance were found in the negatively stained preparation (Fig. 2), but a surface of crisscross pattern was prominent, as observed in paravaccinia virus by Peters et al. [6].

For the serological examination, agar gel precipitation test was conducted using either a convalescent serum from experimentally infected sheep with Orf virus or a hyperimmunized serum against the virus. An ether-treated purified virus was used for the antigen. A common antigen was demonstrated between the antigen prepared from our isolate and that prepared from Orf virus infected cultures. For the serological survey, a total of 78 sera were collected from sheep aging from 2- to 5-year-old in the area where the ovine Orf virus had been isolated. Results showed that more than 25% of sera were positive. The positive reaction was also observed in sera collected in 1970. These results suggest that the disease existed in the area for many years and appeared to be rather widely spread.

In conclusion, the widespread existence of contagious eczema of sheep was proven in the sheep flocks in Hokkaido. The economical importance of the disease seems to be limited. Although the ovine Orf virus isolated was originated from cultured tumor materials, it appeared to be only a
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passenger virus present in the tumor.

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References


Explanation of Figures

Fig. 1. Thin section of co-cultivated cells (tumor cells and sheep choroid plexus cells) on 10th day after cultivation. The mature (M) and immature (I; 260 nm in diameter) particles are seen in the cytoplasm. †: Micelles (60 nm in diameter). Uranyl acetate and lead citrate staining. ×52,000.

Fig. 2. Fine structure of mature virus particles obtained from infected culture. Crosscross pattern of surface filaments is prominent. Silicotungstic acid staining. ×170,000.