Electron Microscopy on Mucosal and Cutaneous Lesions in Contagious Papular Dermatitis of Japanese Serow (Capricornis crispus)

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An electron microscopic study was performed on mucosal and cutaneous lesions of eight Japanese serows infected with contagious papular dermatitis virus. A slight infiltration of lymphocytes and histiocytes, but no virion were seen in the germinal layer and the lower prickle-cell-layer. The cytoplasm was edematous around the nucleus in the cells of the middle prickle-cell-layer. There were many mature viral particles and viroplasm which consisted of electron opaque granular matrix and immature particles in the cytoplasm. The mature particles were oval to cylindrical in shape and measured 300 by 160 nm in size. Tubular structures and filamentous materials were observed in the nuclei of the infected cells. Perinuclear edema spread over in the whole cytoplasm and cytoplasmic organelles were obscure in the cells of the upper prickle-cell-layer. There were numerous mature particles in the cytoplasm, and occasionally such structures as long band-like or spherical accumulations of microgranules were accompanied. It was concluded that papular lesions were caused by viral replication in the cells of the prickle-cells-layer.—Key words: Contagious papular dermatitis, Parapox virus, Japanese serow.


Histopathologic studies on contagious papular dermatitis (CPD) in Japanese serow (Capricornis crispus) were reported in the previous studies [14]. Electron microscopic studies on the lesions of CPD were published in sheep [9, 10, 17] and man [13, 21]. Kumagai et al. [11] and Takatori et al. [19] reported virological findings on the disease in Japanese serow. The purpose of the present study is to clarify the fine structures of papular lesions of CPD in Japanese serow.

MATERIALS AND METHODS

Eight (Nos. 3, 6, 7, 10–14) of 14 Japanese serows in the previous studies [14] were mainly examined for electron microscopy. Tissues taken from the papular lesions were fixed with 6.25% glutaraldehyde in 0.1 M cacodylate buffer at pH 7.4 for 2 hrs at 0°C. Some specimens were prepared from the paraffin blocks corresponding to the papular lesions in the hematoxylin and eosin stained sections after removing the paraffin with xylene. Both specimens were post-fixed with 1% osmium tetroxide at pH 7.3 for 1 hr at 0°C. After dehydration through ethanol series, they were embedded in Epon 812. Ultrathin sections were made and stained with uranyl acetate and lead citrate, examined with Hitachi HS-8 type electron microscope at 50 kv. Successive sections about 1μm were stained with toluidine blue for light microscopy.

RESULTS

On light microscopy, the papular lesions consisted of acanthosis and vacuolar or reticular degeneration of the prickle cells in the upper layer. Each of the prickle cells was swollen than usual. Cytoplasmic inclusions were seen as described previously [14]. The

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horney and granular layers were necrotic and covered with scabs.

On electron microscopy, the basement membrane was well maintained. The germinal cells attached with 10–20 desmosomes each other in the papular lesions. A slight infiltration of lymphocytes and histiocytes was observed. No virion was seen in the cytoplasm of the germinal cells.

The prickle cells of the lower layer were swollen, and the cytoplasm contained bundles of tonofibrils, free ribosomes, and electron opaque keratohyalin granules. Intercellular spaces were extremely narrow and many desmosomes were recognized (Fig. 1). A mass of granular matrix was frequently observed among tonofibrils in the cytoplasm (Fig. 2).

In the prickle cells of the middle layer, edema was obvious around the nucleus in the cytoplasm. The outline of the nucleus was irregular in shape and the chromatin was marginated. One or two inclusions which were composed of granular matrix with immature viral particles were observed in the cytoplasm (Fig. 3). The immature particles were measured 250 to 300 nm in diameter and consisted of amorphous core-like structure surrounded by circular or arch-shaped membranes (Fig. 4). Mature viral particles encircled by membranes were demonstrated (Fig. 5). Mature particles occasionally scattered in the cytoplasm and even in the intercellular spaces. The structure was typical of parapoxvirus and measured 300 by 160 nm in size. Two intranuclear structures were observed in the nuclei of infected cells with virions. One of them appeared to be tubular structure of 100 to 130 nm in the outer diameter and 50 to 80 nm in the inner diameter. Another was bundles of filaments.
Fig. 3. Viroplasm (V) with viral particles is seen in the edematous cytoplasm of the cell at the middle prickle-cell-layer. K: keratohyalin granule. ×5,380.

Fig. 4. Many viral particles at various stages of development are demonstrated in the cytoplasm. ×15,260.

Fig. 5. Mature viral particles enclosed by or released from the shell (arrow) are seen. ×20,790.

Fig. 6. Bundles of filaments (thick arrow) and tubular structures (thin arrows) are seen in the nucleus. ×20,790.
In the prickle cells of the upper layer, the whole cytoplasm was edematous. Disappearance of cytoplasmic organelles was observed. Scattered mature viral particles and granular matrix containing mature and immature viral particles were recognized in the cytoplasm (Fig. 7). An extending band-like structure measured about 300 nm in width, and spherical structure with 1,500 nm in diameter both of which consisted of granules approximately 15 nm in diameter and accompanied with numerous mature viral particles (Figs. 8 and 9). The cellular attachment was well maintained and the intercellular spaces were extremely narrow.

The granular and horny layers were hardly demonstrable.

**DISCUSSION**

On light microscopy, the primary reaction was acanthosis and infiltration of mononuclear cells in the dermis [14, 21]. Although
the virion could not be seen in the germinal layer of the papular lesions, a slight infiltration of lymphocytes and histiocytes was observed. The cellular reaction might be of initial and due to viral infection.

Cytoplasmic changes of the prickle cells were similar to those in bovine papular stomatities (BPS) described by Reczko [18], and in CPD of sheep by Kluge et al. [9] and Knocke [10].

The first alteration was thought to be edema and occurrence of a mass of granular matrix in the epithelial cells of the lower and middle prickle-cell-layers. The matrix contained immature viral particles and coincided with so-called viroplasm [2, 15, 22] and may correspond to the B-type inclusions described by Kato et al. [8]. The matrix was similar to that described by Dales [3] as a large mass of viral deoxyribonucleic acid (DNA) and protein which consisted of tightly grouped 20Å microfibrils, detected in the cells experimentally infected with vaccinia virus.

The membranes surrounding the immature and mature viral particles may correspond to the "shell" described in vaccinia virus [4], Yaba poxvirus [20] and BPS virus [15]. Since the shell was observed as an independent structure and not always associated with mature particles, it would not be the component as already described by Okada and Fujimoto [15].

It seemed that the intranuclear structures corresponded with the intranuclear inclusions observed under light microscopy described in the previous studies [14], and with those in BPS described by Okada and Fujimoto [15], and in cells infected with BPS or orf in vivo as well as in vitro by Pospischil and Bachmann [17]. Fine filamentous networks were also described in swine pox [2] and Junco pox virus infection [1]. Since the intranuclear structures are observed at 16 and 36 hrs post infection in cell cultures infected with BPS or orf virus [17], they may be a secondary product due to viral replication. Recently many workers had reported the synthesis of viral DNA in the nuclei infected with vaccinia and fowlpox viruses by hybridization techniques [5, 7, 12]. Hardy et al. [6] had detected a new protein in the nucleus infected with fowlpox virus, and Pennington and Folley [16] had obtained no evidence for the production of vaccinia virus in enucleated cells. However, it is still unknown whether intranuclear synthesis of viral DNA is concerned with the intranuclear structures or not.

A long band-like or spherical accumulation of microgranules in the cytoplasm of the cells of upper prickle-cell-layer may correspond to the A-type inclusion [8]. This type of matrix was also reported in BPS [15]. Since this structure was observed only in the cells with viral replication, the matrix might have some relations to viral multiplication.

From the present observation we have concluded that papular lesions were caused by intracellular viral replication in the prickle-cell-layer.

REFERENCES

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ニホンカモシカ (Capricornis crispus) の伝染性丘疹性皮膚炎例の粘膜および皮膚病変の電子顕微鏡的検索：岡田洋之・岡田幸助・沼宮内茂・大島寛一（岩手大学農学部獣医学科家畜病理学教室）——ニホンカモシカの伝染性丘疹性皮膚炎 8 例の粘膜および皮膚病変を電子顕微鏡的に検討した。粘膜層および有棘層下層では一部に軽度の細胞浸潤が認められたが、これらの細胞ではウイルス粒子は観察されなかった。有棘層中層の細胞では、細胞質はやや水腫性となり、水腫は核周辺部を中心に拡散する傾向が見られ、核周囲に未成熟ウイルス粒子を含む電子密の高い基質からなるピロプラズマと、その周囲に多数の 300×160 nm の回転棒円体成熟粒子が観察された。核内には管状構造物あるいは線維状物が見られた。有棘層上層の細胞では水腫は細胞質全域におよび、細胞内小器官の消失が著しく、多数のウイルス粒子が観察された。一部の例では細胞質内に帯状あるいは球状の散細顆粒の集合体が観察された。肉眼的あるいは光顕的に認められた丘疹性病変は有棘細胞におけるウイルス増殖にもとづくことが明らかにされた。