The Ultrastructure of Canine Cutaneous Papilloma

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SUMMARY

The fine structure of cutaneous papilloma in the dog was studied in two biopsy specimens fixed in formalin, postfixed in osmium tetroxide, and processed through the Epon embedding technic. The most prominent changes were found in the nuclei of epidermal cells, especially in the granular layer of neoplastic fronds. The affected nuclei contained inclusion bodies in various stages of morphologic differentiation. The inclusions consisted essentially of aggregates of virus particles embedded in the nucleoplasm. The aggregates were often in the form of close-packed crystalline arrays. The individual virus particles in such arrays had a hexagonal profile and measured 450 Å to 490 Å in diameter. In some aggregates, approximately 10 percent of the particles were devoid of the core. These observations present the first morphologic evidence of association of a viral agent with canine cutaneous papilloma. The virus particles found in neoplastic cells had morphologic characteristics indistinguishable from those shown by papilloma viruses.

INTRODUCTION

Although cutaneous papilloma in the dog is not uncommon, there is a dearth of information concerning the structure and etiology of this tumor. The papilloma of the skin in the dog is to be distinguished from oral papilloma, for which viral causation has long been established (6, 7). The etiologic relationship between the cutaneous and oral papillomas has not been determined. The virus of canine oral papilloma is known to possess a high degree of host and tissue specificity, and previous attempts to establish the virus in the skin of experimental animals or tissue culture systems have been unsuccessful (3, 4, 6). Recently, however, Ajello and Gimbo (1) have reported on cutaneous transmission of canine oral papilloma in one of three experimentally inoculated dogs.

The purpose of this communication is to describe the fine structure of the naturally occurring cutaneous papilloma in the dog and to present evidence for the possible association of this type of tumor with a viral agent morphologically indistinguishable from papilloma virus.

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MATERIALS AND METHODS

This study is based on two biopsy specimens submitted to the Diagnostic and Research Laboratory of the Illinois State Department of Agriculture in cooperation with the College of Veterinary Medicine of the University of Illinois.

One growth (No. G6349) was removed from the skin of a nine-month-old male German shepherd dog. The tumor was located in the region of the posterior abdomen and the adjacent, upper part of the medial aspect of the right rear leg; it consisted of numerous small protuberances, greyish-white in color. The second tumor (No. G9861) was removed from the skin of the left side of the neck of a two-year-old male poodle. The growth was first noticed about two weeks before biopsy. In both instances, the neoplastic tissues were fixed in a 4-percent aqueous solution of formaldehyde. The fixative was prepared from commercially available formaldehyde and was not neutralized.

Pieces of the tumors were processed routinely for histopathologic examination, and small blocks, about 1-2 cu mm, were cut from the specimens held in formalin and postfixed for two hours in a 1-percent aqueous solution of osmium tetroxide buffered with s-collidine. The specimens were dehydrated in ascending grades of ethanol and embedded in Epon 812. Sections were cut with an LKB microtome, mounted on uncoated copper grids, stained with lead citrate according to the method of Venable and Coggeshall (14), and examined with a Hitachi 11B electron microscope.

RESULTS

General Architecture of the Tumors. On light microscopic examination, the neoplasms had morphologic characteristics typical of cutaneous papillomas. The neoplasms were composed of numerous papillary fronds of varying width, and the surfaces of these fronds were clothed with multiple layers of keratinized epidermal cells. The subjacent cellular masses, which formed the main structural element of the tumor, consisted essentially of granular and spinous cells. The cells contained infrequent intranuclear inclusion bodies in various stages of morphologic differentiation. In some of the nuclei, inclusion bodies almost filled the entire nucleoplasm and displaced the chromatin toward the periphery. In other instances, they were represented by densely staining basophilic masses occupying only part of degenerating nuclei.

In addition to the intranuclear alterations, epidermal cells, particularly in the upper, superficial reaches of the neoplastic fronds, showed varying degrees of enlargement and ballooning,
with an appreciable decrease in the staining affinity of the cytoplasm.

**Nuclear Changes.** The structure and organization of the inclusion bodies were clearly revealed on electron microscopic examination. The inclusions consisted essentially of aggregates of virus particles embedded in the nucleoplasm. The aggregates were in the form of either close-packed crystalline arrays, or loose accumulations of particles randomly dispersed in the nuclear matrix. As evidenced by serial sections, the crystalline formations were often space-oriented and possessed a recognizable space lattice. The inclusion body shown in Fig. 1 consists of numerous, small crystalline arrays which abut tightly against each other and almost fill the entire nuclear space. Where the lattice pattern is clearly evident, it appears to be of the body-centered cubic type. The structure of crystalline formations is depicted in greater detail in Figs. 2, 3, and particularly in Fig. 4. The crystalline aggregate shown in Fig. 4 is composed of virus particles arrayed in an ordered, translationally periodic pattern characteristic of the space lattice. The relationship of the particles indicates that each particle is surrounded by four nearest neighbors, suggesting that in a space-oriented lattice the particles have eight nearest neighbors. Such a packing arrangement is consistent with a cubic lattice of the body-centered type. Not all virus aggregates had a packing arrangement of this kind. In a crystalline formation shown in Fig. 2, virus particles are arrayed with translational periodicity characteristic of two types of the cubic lattice, either the body-centered or the simple type. The latter type is predominating.

Concurrently with the aggregation of virus particles, the nuclei of the affected cells underwent profound structural changes. The chromatin accumulated at the periphery of the nucleoplasm, and the nuclear membrane began to disintegrate (Fig. 2). As the latter process progressed, the membrane lost its structural definition and was no longer recognizable (Fig. 3). As a consequence, clumps of chromatin, nucleolar substance, and viral aggregates were released into the cytoplasm (Figs. 3, 4). Crystalline formations of virus particles, however, showed no signs of dissociation.

**Morphology of Virus Particles.** When arrayed in close-packed formations, the individual virus particles had a clearly identifiable hexagonal profile, and ranged from 450 Å to 490 Å in diameter. The majority of the particles, however, measured 450 Å. Their center-to-center spacings were estimated at 530 Å. Virus particles consisted of a dense core, approximately 380 Å to 400 Å in diameter, and a readily recognizable delimiting double-contoured membrane, 40 Å thick, which evidently corresponded to the outer shell. In some aggregates, about ten percent of the particles were empty, i.e., devoid of the core. Particles present in loose accumulations tended to have a rounded shape and were approximately of the same size as the particles in crystalline formations.

**DISCUSSION**

The observations described in this report present, for the first time, a morphologic evidence of association of a viral agent with canine cutaneous papilloma. The virus particles found in neoplastic cells possess the morphologic characteristics identical with those shown by papilloma viruses, i.e., (a) size ranging from 450 Å to 490 Å; (b) replication in the nucleus, with the formation of nuclear inclusion bodies; and (c) crystalline mode of aggregation in the nuclear space. On the basis of these characteristics, it would seem reasonable to postulate that the virus described in this communication is probably papilloma virus and may indeed represent the etiologic agent of canine cutaneous papilloma. The exact designation of the virus, however, must await further studies, particularly the experimental transmission of the tumor and reisolation and characterization of the virus.

The postulate receives further support from a well-established observation that papilloma viruses in general have a high degree of host and tissue specificity. Such specificity has been repeatedly demonstrated for the virus of canine oral papillomas, as evidenced by the consistent failure to establish the virus in tissues other than the oral mucous membrane and related structures (3, 4, 6, 8). In view of these observations, it would therefore appear that canine oral papilloma virus is distinct from the virus associated with cutaneous papilloma.

Recently, however, Ajello and Gimbo (1) have described the formation of small papilomatous growths in the skin of one of three experimental dogs inoculated intradermally with canine oral papilloma virus. These findings are at variance with the observations of the previous workers. Further studies based on detailed and careful analysis of experimental material are needed to define the exact role of canine oral papilloma virus in the etiology of papillomas and to determine the antigenic relationship between the viruses associated with the two kinds of canine papillomas.

The character of the cellular changes in cutaneous papilloma, morphology of the virus particles, and the mode of the aggregation are strikingly similar to those described in other papillomas. In sectioned and stained preparations, the size of virus particles was estimated at 380 Å for rabbit oral papilloma virus (11, 12), 460 Å (16) and 380 Å (5) for human wart virus, 420 Å (9) and 250 Å to 350 Å (10) for Shope papilloma virus, 400 Å for bovine papilloma virus (2), and 490 Å for canine oral papilloma virus (15). When measured as center-to-center spacings in close-packed crystalline arrays, the diameter of virus particles was established at 490 Å in rabbit oral papilloma (11) and at 530 Å in canine cutaneous papilloma. The apparent discrepancy in the estimated size of the same virus may be due not only to the effects of fixation, but also to differences in the electron density of viral components. In sectioned and stained preparations, the core of the virus particles often has a density much greater than the shell, and it is therefore more clearly defined.

Papilloma viruses have a propensity to aggregate in crystalline formations in vivo as has been described in rabbit oral papilloma (11, 12), Shope papilloma (13), human wart (5, 15), and canine oral papilloma (15). Factors known to be essential for such ordered crystalline formations are: (a) uniformity in size and shape of individual particles, (b) equivalence of the surface configuration, and (c) relative purity of the aggregates. Since the crystalline state is one of lesser energy, it tends to develop whenever optimal conditions prevail.

The specimens used in this study were fixed in a 4-percent
solution of formaldehyde and kept in it for at least 10 days before they could be postfixed in osmium tetroxide for electron microscopy. In spite of the prolonged storage in unneutralized formalin, the preservation of structural details seemed to be excellent.

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REFERENCES


Fig. 1. An intranuclear inclusion body consisting of numerous close-packed aggregates of virus particles. X 29,500.
Fig. 2. Arrays of virus particles in the nucleus. Clumps of chromatin are present at the periphery. The nuclear membrane shows signs of fragmentation. X 30,500.
Fig. 3. Crystalline formations of virus particles remain in a disintegrated nucleus. A clump of chromatin can be seen at the lower left. X 27,900.
Fig. 4. A close-packed crystalline array of virus particles having a packing arrangement suggestive of the body-centered cubic lattice. X 90,000.
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