Pulmonary Lesions Produced by Fibroma Viruses in Squirrels and Rabbits

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In 1953, Kilham and his associates described naturally occurring fibromas related to Shope's rabbit fibroma in gray squirrels (Sciurus carolinensis). The methods of preparation and passage of the squirrel fibroma virus and the performance of neutralization tests were described (17).

Subsequently, passage of the virus in suckling squirrels was accomplished, and some unusual pulmonary lesions were found in one of the suckling squirrels 50 days after inoculation (15). Grossly, the lungs contained many "pearl-like" lesions 3-4 mm. in diameter. Histologically, these lesions were epithelial rather than fibromatous and resembled pulmonary adenomatosis as seen in man and some other animal species.

We have recently had the opportunity to examine the lungs of two other suckling squirrels inoculated with the squirrel fibroma virus, and the adenomatosis-like lesions were again found. This report describes the histopathology of these lesions and presents some additional experimental observations on pulmonary lesions produced by fibroma viruses in squirrels and rabbits.

MATERIALS AND METHODS

Squirrels.—Wild gray squirrels were trapped in Maryland. Females found to be pregnant were kept in small cages. Methods of handling suckling squirrels and adult squirrels have been described previously (14).

Rabbits.—Suckling rabbits were from mothers of unknown ancestry purchased from local dealers.

Mice.—CSH mice of the Heston subline were obtained from the animal colony of the National Institutes of Health.

Hamsters.—Golden hamsters were obtained from the animal colony of the National Institutes of Health.

Viruses.—The squirrel fibroma virus was originally isolated in 1953 and propagated in suckling squirrels and in young woodchucks (Marmota monax) (17). The squirrel fibroma virus was also propagated in tissue cultures of tryptone-digested rabbit kidney and squirrel kidney. Fourteen passages were carried out in both types of culture. Tests for the presence of virus were made by intracutaneous inoculation of adult rabbits, and a pool of the tenth- and twelfth-passage fluids from squirrel kidney cultures had a virus titer of $10^{-3}$. Further details on tissue culture methods are given in a previous publication (16).

The Shope rabbit fibroma virus used in these studies was the Patuxent strain and was propagated in rabbit kidney cell cultures according to methods previously described (16).

METHODS OF INOCULATION.—Intratracheal inoculations were accomplished in squirrels and rabbits under ether anesthesia by first incising the skin and exposing the trachea and then injecting 1 or 2 ml. of virus suspension with a syringe and No. 26 gauge needle. Methods of intradermal and subcutaneous inoculation of suckling squirrels have been previously described (15).

RESULTS

Intracutaneous inoculation of squirrel fibroma virus in suckling squirrels.—Squirrel 1 was born on March 25, 1957, and inoculated intracutaneously on the back with squirrel fibroma virus 2 days after birth. Twelve days after inoculation, a mass was present at the site of inoculation, and this rapidly increased in size with the appearance of satellite nodules in the adjacent skin. Twenty-two days after inoculation this baby squirrel was moribund and was sacrificed.

At autopsy, in addition to the masses in the skin and subcutis, there were multiple gray-white nodules measuring 1-2 mm. in diameter in the lungs. There was also a small, gray-white lesion in the medulla of one kidney. No other lesions were noted grossly. Portions of the skin mass, the lungs, and the involved kidney were fixed in 10 per cent buffered formalin for histologic studies. Unfortunately, no other tissues were saved for histologic examination. Squirrel fibroma virus was isolated from the lung nodules and from the kidney lesion by inoculation of young woodchucks. Virus from both sources produced typical fibromas in the young woodchucks. Squirrel fibroma virus was also isolated from the pulmonary and renal lesions by inoculation of squirrel kidney tissue cultures.

Microscopically, the tumor mass in the skin and subcutis was similar to squirrel fibroma lesions previously described (17) and resembled the Shope fibroma lesion in rabbits (Fig. 1). The renal lesion noted grossly consisted of a proliferation of "fibroma cells" in the interstitial tissue of the medulla of the kidney.
In the lungs, there was a large area in which the alveoli were lined by tall columnar epithelial cells, resulting in a histologic appearance strikingly similar to lesions described as pulmonary adenomatosis in man. The involved area was rather well demarcated from the adjacent pulmonary parenchyma, and several small bronchioles were included in the lesion (Fig. 2). The tall columnar cells lining the pulmonary alveoli had basal nuclei and faintly eosinophilic cytoplasm (Fig. 3). In a number of these columnar cells, large eosinophilic intracytoplasmic inclusion bodies similar to those seen in the squamous epithelium of the cutaneous lesion were present (Fig. 4). In several areas, small bronchioles could be seen with areas of transition from the normal pseudo-stratified, ciliated respiratory epithelium to the clear columnar cells with inclusion bodies of the adenomatosis-like lesion (Fig. 5). Sections prepared with the periodic acid–Schiff reaction after diastase digestion showed a material with the characteristics of mucin in the alveolar lumina and within some of the columnar cells (Fig. 6).

Approximately 1 year after the first suckling squirrel (squirrel 1) with the adenomatosis-like lesion had been examined, another pregnant squirrel was trapped. Two squirrels were born on February 27, 1958, and inoculated, on the day of birth, intratracheally with squirrel fibroma virus. Both developed large masses at the inoculation sites. One of the baby squirrels died and was eaten by the mother 20 days after inoculation. The other, squirrel 2, was moribund 22 days after inoculation and was sacrificed. Grossly, in addition to the large mass at the inoculation site with small adjacent satellite skin nodules (Fig. 7), there were multiple 1–2-mm. nodules in the lungs. The largest of these was similar histologically to the adenomatosis-like lesion seen in squirrel 1 (Fig. 8). In some of the other lung nodules, in addition to columnar cells lining alveoli, proliferation of elongated “fibroma cells” from the adventitia of vessels and the walls of small bronchi was prominent.

There were also fibroma lesions in the liver and the kidney. The renal lesion is of some special interest in that there was a proliferation of tubular epithelial elements as well as stromal cells. Occasional eosinophilic intracytoplasmic inclusion bodies were seen in the epithelial cells lining the tubules in this lesion.

Squirrel fibroma virus in suckling rabbits.—Because of interest in the adenomatosis-like lesion of the suckling squirrels and because of difficulty in obtaining sucking squirrels, a series of experiments was undertaken on squirrel fibroma virus in suckling rabbits shortly after birth. Virus was inoculated intradermally and subcutaneously in two litters of rabbits on the day of birth, and these animals were sacrificed 10–14 days later. No lesions were found. Subsequently, two litters of rabbits were inoculated with squirrel fibroma virus intratracheally. Twelve animals survived this procedure and remained well. When examined at autopsy, 14 days after inoculation, no lesions were found.

Squirrel fibroma virus in suckling mice and hamsters.—Squirrel fibroma virus was inoculated subcutaneously into three litters of C3H mice and three litters of golden hamsters on the day of birth. Some of the suckling mice and hamsters died within 1 week of inoculation. No fibromas at the inoculation sites and no visceral lesions were found in these animals. The surviving mice and hamsters are alive 1 year after inoculation with no evidence of lesions produced by fibroma virus.

Subcutaneous inoculation of Shope rabbit fibroma virus in suckling rabbits.—Two litters of rabbits were given subcutaneous inoculations of the Shope fibroma virus on the day of birth. The animals all developed large masses at the inoculation sites and died within 7–12 days. At autopsy they had extensive visceral involvement by fibroma lesions, resembling those described by Duran-Reynals in similar experiments (7). In the lungs, a number of small peripheral lesions were found, consisting predominantly of proliferation of fibroma cells in the alveolar septae. Some of the adjacent alveoli were lined by cuboidal epithelial cells, but the lesions did not resemble the adenomatosis-like lesions produced in the sucking squirrel by squirrel fibroma virus. There were also some large fibroma lesions around large pulmonary blood vessels, as previously described by Duran-Reynals (7), and similar lesions were seen around bronchi.

Intratracheal inoculation of Shope rabbit fibroma virus in suckling rabbits.—Three litters of rabbits were given inoculations of Shope fibroma virus intratracheally 1–4 days after birth. All these animals developed respiratory difficulty and died 6–12 days after inoculation.

At autopsy, all had extensive involvement of the trachea and surrounding tissues at the tracheostomy site by fibroma. There was also involvement of adjacent skeletal muscle, thyroid gland, thymus gland, and esophagus. In the lungs, there were small peripheral lesions similar to those described in the animals inoculated subcutaneously, and there were also perivascular proliferations of “fibroma cells” around large pulmonary blood vessels. Of special interest were the lesions of major bronchi. There were proliferations of bronchial...
columnar epithelial cells, some of which contained intracytoplasmic eosinophilic inclusion bodies and many of the alveoli were lined by cuboidal and large areas of pulmonary edema and pneumonia, killed. At autopsy, there was a severe necrotizing pneumonitis but no adenomatosis-like lesions. At intratracheal inoculation and had areas of necrotizing pneumonitis but no adenomatosis-like lesions. Intratracheal inoculation of squirrel fibroma virus in squirrels.—In October of 1957, a young squirrel (squirrel 3) was trapped and subsequently squirrel fibroma virus was inoculated intratracheally. The animal survived the procedure and did well for 11 days. On the 12th day after inoculation, tachypnea and dyspnea were present, and the animal was killed. At autopsy, an acute necrotizing bronchopneumonia was present, and, microscopically, large numbers of neutrophilic leukocytes were seen in bronchi and alveoli. There were also large areas of pulmonary edema. In some areas, alveoli were lined by cuboidal epithelial cells which contained large, intracytoplasmic, eosinophilic inclusion bodies (Fig. 12).

Subsequently, fibroma virus was inoculated intratracheally into nine adult squirrels (squirrels 4 through 12).

Squirrel 4 developed signs of respiratory distress 18 days after intratracheal inoculation and was killed. At autopsy, there was a severe necrotizing pneumonitis with some cuboidal cells lining alveoli, but no adenomatosis-like lesions similar to those seen in squirrels 1 and 2 were present.

Squirrel 5 was found dead 16 days after intratracheal inoculation. At autopsy, a number of small gray-white glistening lesions were seen grossly in the lungs. Histologically, in such areas, there was marked papillary proliferation of bronchial epithelium (Fig. 13), with eosinophilic inclusion bodies present in the epithelial cells (Fig. 14). Proliferation of spindle-shaped "fibroma cells" around blood vessels and bronchi was seen. There were large areas of pulmonary edema and pneumonia, and many of the alveoli were lined by cuboidal and columnar epithelial cells, some of which contained intracytoplasmic eosinophilic inclusion bodies.

Squirrel 6 was sacrificed 18 days after intratracheal inoculation and had areas of necrotizing pneumonitis but no adenomatosis-like lesions.

Squirrels 7–12 were given intratracheal inoculations of squirrel fibroma virus but remained well.

When sacrificed, 18–26 days after inoculation, no significant pulmonary lesions were found.

**DISCUSSION**

Pulmonary adenomatosis has been described in man (23), sheep (8, 8), mules (24), horses (24), guinea pigs (11), mice (19), chinchillas (12), and a number of other animal species (8). The question whether these lesions are neoplasms or reactive processes has not been answered conclusively. Swan, in his review of pulmonary adenomatosis in man, states that the "majority of observers are of the opinion that pulmonary adenomatosis is an extrabronchial neoplasm with cancerous potentialities" (23).

The etiology of pulmonary adenomatosis in man is unknown. Pulmonary adenomatosis in sheep has been extensively studied, and a recent review by Duran-Reynals and his associates describes the pulmonary adenomatosis complex of sheep in detail (8). The disease in sheep has occurred in both epizootic and enzootic form and has been thought to be an infectious disease caused by a virus. Experiments in which attempts have been made to transmit the disease have been described by Dungal (5, 6) and Duran-Reynals (8), and the difficulties in interpretation of such experiments have been discussed. The isolation of a virus which induces the adenomatous lesions in the lungs of sheep has not yet been conclusively demonstrated.

In DBA mice, pulmonary adenomatosis has been induced by ingestion of suspensions of 1,2,5,6-dibenzanthracene and 20-methylcholanthrene (18). De Kock, however, was unable to produce the lesions in sheep with carcinogenic hydrocarbons (4).

The pulmonary adenomatosis-like lesions of the squirrel which we have described seem to be causally related to the squirrel fibroma virus. The columnar cells lining the alveoli contain large eosinophilic intracytoplasmic inclusion bodies, and the squirrel fibroma virus has been recovered from the lesions. This is the first example of established viral etiology of a lesion closely resembling pulmonary adenomatosis of man.

With regard to the proliferative lesions of the bronchial epithelium seen in squirrel 5 and in some of the suckling rabbits, a report published by Roux in 1903 is of considerable interest (19). He described adenomatous proliferative lesions of the bronchial epithelium of sheep produced by the sheep pox virus. He concluded that "this action of the sheep pox virus on epithelia is common to it and to the still unknown cancer agent; so that if the sheep pox virus falls into the class
of extremely small microbes, the etiologic agent of cancer may also someday find its place there.”

The production of epithelial lesions in the lung by the same virus that causes typical fibromas in the skin is of interest in relation to recent work on viruses and cancer. Hyperplasia of the stratified squamous epithelium of the skin overlying the Shope rabbit fibroma was described by Shope (20) and by Ahlström (1). Duran-Reynals noted proliferation of bile duct epithelium in areas of fibroma in the livers of suckling rabbits given inoculations of the rabbit fibroma virus (7). Carr has described papillary epithelial lesions in the kidneys of young chicks given inoculations of the ES4 strain of Engelbreth-Holm erythroleukemia virus (9). He diagnosed these lesions as renal adenocarcinomas and found that their production was facilitated by direct inoculation of the virus to the kidneys. Rubin has studied the focal lesions of the choioallantoic membrane of the embryonated chick egg given inoculations of Rous sarcoma virus and has described proliferation of both epithelial and connective tissue elements (20). Both Gross (10) and Stewart (22) have described tumors of the parotid glands of mice apparently induced by a virus, and, microscopically, these tumors are pleomorphic with both epithelial and mesenchymal elements. Preparations of the same agent also induce a variety of other neoplastic lesions, some of which are sarcomatous, in mice. Eddy and her associates have recently been able to produce sarcomatous lesions in young hamsters with the same tissue culture preparations that produce the pleomorphic salivary gland tumors in mice (9).

The problem of whether the pulmonary adenomatosis-like lesions of the squirrel induced by squirrel fibroma virus are viral proliferative lesions or neoplasms caused by the virus remains unsettled. Attempts to transplant the lesions should be undertaken, but this has thus far not been possible because of the difficulty of obtaining sucking squirrels. It seems probable, however, that subcutaneous transplantation of the lung lesions that are known to contain virus would result in the production of a fibroma at the transplantation site and, perhaps, in generalized disease in the suckling squirrel. It would also be desirable to attempt to transplant the pulmonary lesions to squirrels immunized against squirrel fibroma virus, either actively or passively, to determine whether the lesion is indeed dependent upon the agent. Because there is no genetic homogeneity in wild squirrels, negative results, however, would not be proof that the lesion is not an autonomous neoplasm.

Although a decision regarding the neoplastic or proliferative nature of these lesions is important, it must be remembered that a sharp separation of proliferative lesions from neoplasms is often extremely difficult. Now that tissue culture and other precise methods for study of viruses are readily available, investigations of the mechanisms by which viruses induce cellular proliferation may be possible. The squirrel fibroma virus may be well suited for such work, and the fact that this agent induces a lung lesion resembling pulmonary adenomatosis in man adds to the value of studies made with it.

SUMMARY

Lesions resembling pulmonary adenomatosis have been produced in sucking squirrels given inoculations of squirrel fibroma virus. The columnar cells lining the pulmonary alveoli in the involved areas have large eosinophilic intracytoplasmic inclusion bodies, and squirrel fibroma virus has been recovered from these lesions. Attempts to reproduce the lesion in sucking rabbits, hamsters, and mice with the squirrel fibroma virus have been unsuccessful. Proliferative lesions of both bronchial epithelium and the fibromuscular tissue of the bronchial wall have been produced in sucking rabbits after intratracheal inoculation of Shope rabbit fibroma virus, and similar proliferative pulmonary lesions have been produced in some adult squirrels after intratracheal inoculation of squirrel fibroma virus. The importance of the route of inoculation of these viruses in the determination of the pathologic lesions produced has been demonstrated, and the capacity of these agents to produce proliferation of epithelial cells in the lung has been clearly shown.

The demonstration of a viral lesion resembling pulmonary adenomatosis in squirrels suggests that a more intensive search for a viral etiology of pulmonary adenomatosis in man should be undertaken.

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REFERENCES


FIG. 1.—Cutaneous fibroma in suckling squirrel (Squirrel 1) 22 days after intradermal inoculation of squirrel fibroma virus. There are large eosinophilic intracytoplasmic inclusion bodies in the cells of the surface stratified squamous epithelium, and there is a proliferation of "fibroma cells" in the corium. Inclusion bodies are indicated by arrows. Hematoxylin & eosin, ×100.

FIG. 2.—Lung of suckling squirrel (Squirrel 1) 22 days after intradermal inoculation of squirrel fibroma virus. The large area of involvement resembles pulmonary adenomatosis. Several small bronchi are included in the lesion. Hematoxylin & eosin, ×11.
FIG. 3.—Lung lesion in suckling squirrel (Squirrel 1) 22 days after intradermal inoculation of squirrel fibroma virus. The alveoli are lined by columnar cells with basal and miderional nuclei. Intracytoplasmic inclusion bodies can be seen in some cells. Hematoxylin & eosin, X480.

FIG. 4.—Lung lesion in suckling squirrel (Squirrel 1) 22 days after intradermal inoculation of squirrel fibroma virus. Large eosinophilic cytoplasmic inclusion bodies (indicated by arrows) are present in the columnar cells lining the alveolar spaces. Eosinophilic material resembling mucin is present in the lumen. Hematoxylin & eosin, X800.
Fig. 5.—Lung lesion in suckling squirrel (Squirrel 1) 22 days after intradermal inoculation of squirrel fibroma virus. A small bronchiole lined by pseudostratified ciliated columnar epithelium is seen, with areas of transition to the adenomatosis-like lesion. Hematoxylin & eosin, X360.

Fig. 6.—Lung lesion in suckling squirrel (Squirrel 1) 22 days after intradermal inoculation of squirrel fibroma virus. Alveoli are lined by columnar cells. Material with the histochemical characteristics of mucin is seen in the alveoli. Arrow indicates inclusion body. Periodic acid-Schiff reaction preceded by diastase digestion. X410.
FIG. 7.—Suckling squirrel (Squirrel 2) 22 days after intradermal inoculation with squirrel fibroma virus. There is a tumor mass at the site of inoculation with some necrosis and hemorrhage on the surface. There are several small satellite skin nodules.

FIG. 8.—Lung lesion of suckling squirrel (Squirrel 2) 22 days after intradermal inoculation of squirrel fibroma virus. A small bronchus entering an area involved by the adenomatosis-like lesion is seen. An inclusion body is indicated by an arrow. Hematoxylin & eosin, ×410.
Fig. 9.—Lung lesion of suckling rabbit 14 days after intratracheal inoculation of Shope rabbit fibroma virus. There is proliferation of bronchial epithelium and marked proliferation of elongated “fibroma cells” which appear to arise in the fibromuscular tissue of bronchial wall. Hematoxylin & eosin, ×60.

Fig. 10.—Lung lesion of suckling rabbit 14 days after intratracheal inoculation of Shope rabbit fibroma virus. Proliferation of the bronchial epithelium can be seen. Hematoxylin & eosin, ×830.
FIG. 11.—Lung lesion of suckling rabbit 12 days after intratracheal inoculation of Shope rabbit fibroma virus. At the margin of the large fibroma lesion shown in Figure 9, some alveoli are lined by cuboidal cells and contain mucin. Periodic acid-Schiff reaction preceded by diastase digestion. ×850.

FIG. 12.—Lung lesion of young squirrel (Squirrel 8) 12 days after intratracheal inoculation of squirrel fibroma virus. The alveoli are lined by cuboidal cells, some containing large eosinophilic intracytoplasmic inclusion bodies (indicated by arrow). Hematoxylin & eosin, ×850.
FIG. 13.—Lung lesion of adult squirrel (Squirrel 5) 16 days after intratracheal inoculation of squirrel fibroma virus. There is marked papillary proliferation of bronchial epithelium. On the left side of the photograph, alveoli lined by cuboidal epithelial cells can be seen. Hematoxylin & eosin, ×115.

FIG. 14.—Lung lesion of adult squirrel (Squirrel 5) 16 days after intratracheal inoculation of squirrel fibroma virus. This is a higher magnification of the papillary areas of bronchial epithelium seen in Figure 13. Large eosinophilic intracytoplasmic inclusions (indicated by arrow) are present in the epithelial cells. Hematoxylin & eosin, ×780.
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