Advances in Brief

Postvaccinal Sarcomas in the Cat: Epidemiology and Electron Probe Microanalytical Identification of Aluminum

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Abstract

An increase in fibrosarcomas in a biopsy population of cats in the Pennsylvania area appears to be related to the increased vaccination of cats following enactment of a mandatory rabies vaccination law. The majority of fibrosarcomas arose in sites routinely used by veterinarians for vaccination, and 42 of 198 tumors were surrounded by lymphocytes and macrophages containing foreign material identical to that previously described in postvaccinal inflammatory injection site reactions. Some of the vaccines used have aluminum-based adjuvants, and macrophages surrounding three tumors contained aluminum oxide identified by electron probe microanalysis and imaged by energy-filtered electron microscopy. Persistence of inflammatory and immunological reactions associated with aluminum may predispose the cat to a derangement of its fibrous connective tissue repair response, leading to neoplasia.

Introduction

The Laboratory of Pathology of the University of Pennsylvania School of Veterinary Medicine has for the past 20 years provided a surgical pathology service to veterinarians predominantly in the mid-Atlantic states (Pennsylvania, New Jersey, and Maryland). Approximately 17,500 total accessions are received per year, 20–30% of which are from cats. Beginning in 1987, we noted an increase in the number of cases of s.c. inflammatory injection site reactions in cats, a diagnosis that had been previously made only rarely in our biopsy service. The number of these reactions increased yearly, totalling 75 by the end of 1991. This increase coincided with the enactment of the 1987 Pennsylvania state law requiring rabies vaccination of cats. Between 1987 and 1991, there has also been a 61% increase in the number of fibrosarcomas in our feline biopsy accessions. Epidemiological, histological, and ultrastructural evidence, presented below, suggest that these two phenomena are related.

Materials and Methods

All case material was received through the biopsy service of the School of Veterinary Medicine at the University of Pennsylvania. Specimens were fixed in 10% formalin, routinely processed, embedded in paraffin blocks, and stained with hematoxylin and eosin. For transmission electron microscopy, specimens were retrieved from deparaffinized blocks, en bloc stained with uranyl acetate, and reembedded in Spurr’s low viscosity embedding medium. Postfixation with osmium tetroxide was omitted. All specimens were handled with nonaluminum tools to avoid contamination, and were placed on formvar/carbon-coated copper grids. Specimens for electron microscopy were poststained with uranyl acetate/lead citrate; those destined for electron probe microanalysis were left unstained. The details of the principles and methods used for the electron probe analysis have been previously published (1, 2). Briefly, X-ray spectra were collected with a Link Pentafet X-ray detector mounted on a Philips CM12 electron microscope and connected to a Keve7000 multichannel analyzer interfaced to PDP11/34 and VAX 750 computers. The ultrathin window of the Pentafet detector permits the detection of lower atomic number elements than possible with a more conventional detector equipped with a beryllium window and, in the present study, shows the association of oxygen with aluminum.

Energy-filtered (3) scanning transmission electron micrographs were obtained with a Philips EM 400 electron microscope equipped with a field emission gun. First difference energy loss spectra were collected with a parallel detection–magnetic sector electron spectrometer at each pixel and multiple least squares fitted to the plural scattering plasmon/valence spectra and to reference files of aluminum and oxygen (4, 5). Data were analyzed by the $x^2$ test for trend (6).

Results and Discussion

The yearly site distributions of all feline nonoral fibrosarcomas received between January 1, 1987 and December 31, 1991 are listed in Table 1. During this time, the percentage of these tumors in our total feline biopsy population increased from 3.6 to 5.8% ($x^2$ trend = 26.1, $P < 0.0001$), primarily due to increases at selected sites. While the number of fibrosarcomas at noninjection sites stayed essentially the same, there was a significant increase ($x^2$ trend = 11.5, $P < 0.0007$) in the following sites: hindlimb, dorsal neck/scapular, dorsal lumbar, flank, and dorsolateral thorax. These are sites routinely used by veterinarians for s.c. and i.m. vaccinations. Furthermore, the most noticeable increases have been in the dorsal neck/scapula and dorsolateral thorax, typical sites for s.c. vaccination. Vaccination i.m. for rabies was the only approved method in the United States until 1985 when a rabies vaccine became available that could be administered by either s.c. or i.m. routes. Anecdotal evidence suggests that many veterinarians have switched to the s.c. route of inoculation over the 1985 to 1991 period.

A review of the histology of the fibrosarcomas received in 1991 revealed that 51% (101 of 198) were surrounded and partially infiltrated by lymphocytes and macrophages. In 42 of these tumors (17 dorsal neck/scapular, 10 dorsolateral thorax, 7 dorsal lumbar, 4 thigh, and 4 flank), gray-brown granular to crystalline foreign material was found within macrophages in the inflammatory foci (Fig. 1). Fifteen % (14 of 92) of feline fibrosarcomas received in 1986 were surrounded by a similar inflammatory infiltrate, but none contained the crystalline foreign material.
Table 1  Site distribution of feline fibrosarcomas in biopsy files of the Laboratory of Pathology

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% of total accessions: 3.6, 3.0, 3.8, 4.2

* Includes ventral neck/chest, tail, perianal area, and scrotum.

Fig. 1. Representative light micrograph of a section from the edge of a sarcoma in a vaccination site. Macrophages with wispy to granular foreign material are seen at the top, above a population of lymphocytes. A sclerotic portion of the sarcoma is seen below. H & E. Bar, 50 μm.

Fig. 2. Representative electron micrograph of a macrophage at the edge of a sarcoma in a vaccination site. N, nucleus. The cytoplasm contains large and small aggregates of electron-dense granular material (*), shown to consist of aluminum and oxygen (Figs. 3/4). Bar, 1 μm.

Foreign material identical to that described above has been previously described within macrophages in postvaccinal inflammatory injection site reactions in cats and dogs (7). These inflammatory lesions are quite distinctive and consist of a well-demarcated focus of granulomatous panniculitis, usually with central necrosis and peripheral lymphoid follicles. Ultrastructurally, the material in macrophages surrounding the sarcomas was electron dense, linear to slightly curved, and arranged in lamellar stacks (Fig. 2). Electron probe X-ray microanalysis showed, in each of three sarcomas evaluated, that the material was composed of aluminum and oxygen. Representative X-ray spectra of the material and of the adjacent region (Fig. 3) showed that only the material contained aluminum, and this was associated with a high concentration of oxygen. Aluminum was not detected in the adjacent area. Energy-filtered scanning transmission electron microscopy of...
Fig. 3. Representative X-ray spectra collected from a granular deposit (A) and from adjacent cytoplasm (B). Note the presence of the very large aluminum (Al) and oxygen peaks (O) in the spectrum of the deposit, and the absence of aluminum in the adjacent region. The small oxygen peak in the cytoplasmic area is due to the presence of oxygen in the embedding material and/or cytoplasmic proteins.

Fig. 4. Energy-filtered scanning transmission electron micrographs of a region of a cell containing granular deposits. TL, low magnification dark field scanning transmission electron micrograph (STEM). TR, scanning transmission electron micrograph view and images formed with, respectively, electrons showing the characteristic loss resulting from oxygen K-shell and aluminum L-shell excitation (O and Al images). Since hydrogen is not detectable by either electron probe X-ray microanalysis or electron energy loss spectroscopy, it cannot be determined whether these deposits consist of aluminum hydroxide or aluminum oxide. Electron energy loss spectra (shown below) collected over 1 pixel within the deposit show the characteristic, first-difference oxygen (BL) and aluminum (BR) peaks. Sections were carbon coated to reduce charging of the specimen, giving rise to the carbon K-loss (C) edge in the energy loss spectra. Probe currents (3 s/pixel) for the oxygen map and the aluminum map were 1.8 nA and 0.05 nA, respectively, and the pixel size is 36 nm.

such regions (Fig. 4) also showed the material in both the aluminum and oxygen images.

Aluminum hydroxide gel is the adjuvant in one of the commonly used feline rabies vaccines; however, information on the exact composition of each feline vaccine is very difficult to obtain due to the proprietary nature of this type of information. From 1989 to 1990, aluminum, as aluminum hydroxide or aluminum phosphate, was used as an adjuvant in 19.6% of...
feline vaccine products produced during that period.\(^3\) Aluminum-based adjuvants have been incriminated as the cause of allergic foreign body granulomas in humans (8, 9). Electron-dense material, similar to that described here, was demonstrated in histiocytes in those granulomas and the histology of the foreign body reactions was identical to the injection site reactions in cats and dogs previously reported by one of us (7). The biocompatibility of aluminum in animals has been investigated in rodents and dogs (10), but not cats. In those species evaluated, the only consistent response was inflammation characterized by histiocytes (macrophages) and lymphocytes. An aluminum oxide ceramic hip prosthesis has been incriminated as the possible cause of a soft tissue sarcoma in a 40-year-old man (11), but we have been unable to find any other reports of aluminum-associated neoplasia in animals or humans.

We have information from 34 veterinarians that the sarcomas arose in vaccination sites; however, many veterinarians inject more than one vaccine at one site, and many do not have complete vaccination histories, making the association with a specific aluminum-containing vaccine difficult in the majority of our cases. The interval between vaccination and tumor appearance in the aforementioned 34 cases ranged from 3 months to 3 years. In one case, a sarcoma developed in an area in which a mass had been previously excised and determined histologically to be an inflammatory injection site reaction. Based on available information, our interpretation is that the persistence of the inflammatory and immunological reactions associated with the presence of the aluminum in the injection sites predisposes the cat to a derangement of its fibrous connective tissue repair response, eventually leading to neoplasia in some of these cases. In favor of this hypothesis is the fact that a few cases in our files were "transitional": microscopic foci of sarcoma were found in areas of granulomatous inflammation. There is precedent for this type of oncogenesis in the cat. Sarcomas have been reported to develop in the eyes of cats following persistent or previous trauma (12). The pathogenesis of those sarcomas could be similar to that proposed here. In addition, viruses and/or oncogenes may play a role in the induction of these tumors. Feline sarcoma virus is a retrovirus of cats that possesses transforming oncogenes that can induce fibrosarcomas. However, feline sarcoma virus-induced sarcomas are rare, are usually multicentric, and are typically seen in cats less than 3 years of age (13). (Only 8% of our cats with fibrosarcomas were 3 years old or less.) More extensive and detailed retrospective and prospective epidemiological and virological studies are under way to further evaluate the relationship between vaccination and oncogenesis in the cat. Whether the association of aluminum with feline sarcomas, found in the present study, indicates an oncogenic effect or is merely a "marker" verifying that sarcomas developed at the sites of injection of vaccines in which aluminum hydroxide was used as an adjuvant, remains to be determined.

Acknowledgments

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References


\(^3\) Biologics Summary Production Report, United States Department of Agriculture.
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