Experimental Anterior Uveitis after Subcutaneous Injection of Feline Sarcoma Virus

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Feline sarcoma virus (FeSV) is a naturally occurring virus that causes spontaneous tumors in cats. The immunologic and morphologic characteristics of these tumors have been studied extensively. It was recently observed in experiments undertaken to induce systemic malignancy with this virus, that severe uveitis and clinical blindness occurred. An investigation of the ophthalmologic changes was undertaken. A fulminant anterior uveitis was produced in cats by a series of subcutaneous injections of live FeLV-FeSV. This intraocular inflammation occurred in five of six animals using high viral titers, and four of seven with lower titers, resulting from the freeze thaw process. On histopathologic examination, most animals demonstrated dysplastic changes of the ciliary body in addition to the iridocyclitis. The remainder of the eye was unaffected. These animals developed systemic tumors unaccompanied by local inflammation, many of which spontaneously regressed. Notable features of this potential model for uveitis are that (1) direct injection into the eye is unnecessary, and (2) intravenous administration inducing immune tolerance with antigenic overload presented to the spleen is avoided. This inflammatory reaction seems to be specific to the iris and ciliary body. Levels of live virus detected in the aqueous humor exceeded those in the serum. These results suggest that the virus may be actively secreted by the ciliary epithelium, or may preferentially proliferate within the eye.

Materials and Methods

Six 2- to 3-month-old kittens from the specific pathogen free (SPF) colony at the New York State College of Veterinary Medicine at Cornell University, weighing about 400 g each were inoculated subcutaneously on day 0 and 21, alternating right and left flanks, with 1.0 ml of a suspension containing about 45 picograms of a feline leukemia virus (FeLV) pseudotype of murine sarcoma virus (MuSV). The flanks were clipped prior to inoculation to insure that all inoculum was properly administered in each cat. All
Experimental animals were housed in Horsfall cages in which air was filtered to avoid contamination with extraneous agents.

The pseudotype virus MuSV (FeLV) was obtained from the superinfection of CCC81 cells that contain MuSV® with cell free supernatant from a 36-hour culture of the F422 cell line that had approximately 10^10 particles of FeLV/ml. F422 is a cat lymphoblastoid cell line permanently infected with FeLV, which was derived from a thymic lymphoma induced by the inoculation of FeLV.7 For infection of CCC81, FeLV was allowed to adsorb for 45 min at 37 C in a humidified CO2 incubator. The CCC81 cells were washed extensively with McCoy's 5A media without fetal bovine serum and thereafter maintained 12 days with the same media supplemented with 15% fetal bovine serum and antibiotics. In order to retain as much MuSV (FeLV) as possible, the growth media was not changed. At day 12 about 90% of the cells were morphologically transformed. The supernatant and floating cells were centrifuged for 15 min at 2000 RPM in an IEC refrigerated centrifuge to separate cells and cellular debris from the supernatant. This supernatant, which contained the MuSV (FeLV), was used as inoculum in the experimental animals.

At day 42, 21 days after the second inoculation with MuSV (FeLV) all kittens were challenged with 10^2 LD_{50} of the Snyder-Theilen strain of FeSV(ST-FeSV). Determination of the LD_{50} was previously made in SPF kittens of comparable age and weight.8

These experiments were performed twice. In the first set of six animals uveitis was noted incidentally to the primary purpose of the experiment, which was to produce tumors. So, these animals were not biomicroscopically monitored for the appearance of cells in the anterior chamber. However, when the experiment was later repeated in seven animals, the animals were examined carefully with the slit-lamp biomicroscope before any injections and then every 48 hours during the experiment until evidence of anterior uveitis was found. The same virus preparation was used in both experiments, the only difference being that this preparation had undergone the freeze-thaw process. The animals were killed for histopathology between 2 days and 4 weeks after the appearance of the ocular inflammation.

Virus titrations of peripheral blood and aqueous humor were made by scoring infectious units of FeLV in CCC81 cells and transforming units of MuSV and/or FeSV in CCL64 mink cells.9 These values are averages taken from determinations of samples from each animal which showed the uveitis.

Results

The first animal showed conjunctival injection and ciliary body flush 37 days after challenged with ST-FeSV (Fig. 1, clinical photo). Six days later, three other animals developed the same signs. Ophthalmologic examination revealed photophobia, iritis, posterior synechiae, with pigment deposition on the anterior lens capsule, and cortical cataracts. The ret-
In the fifth animal, mild manifestations of the ocular lesion developed over the next week. One animal never developed the ocular lesion.

On pathologic examination, by light microscopy, the corneal epithelium showed mild microcystic edema, the stroma was intact. There was no interstitial keratitis. The endothelium was cystic and degenerated, but Descemet's membrane appeared normal. The anterior chambers were filled with fibrinous material and inflammatory cells, mainly mononuclear cells: plasma cells, lymphocytes, macrophages. Several sections showed the inflammatory cells to have coalesced on the corneal endothelium, forming keratic precipitates (Fig. 2).

The chamber angle in all specimens was open, although in one case it was filled with inflammatory cells and debris. The iris was infiltrated focally with lymphocytes and plasma cells, and bizarre "transformed" cells appeared at its foot (Fig. 3). In one case the iris vessels were greatly dilated with marginated polymorphonuclear leukocytes (Fig. 4). The pigment epithelium of the iris was, for the most part, intact, but one specimen showed breakthrough of inflammation posteriorly with hemorrhagic congestion and necrosis of pigment and iris tissue.

The ciliary body was the site of the most unusual changes in all specimens. The ciliary musculature was infiltrated focally with lymphocytes, especially the...
outer layers (Fig. 5). The pigmented and nonpigmented epithelia were disrupted, cystic, and pigment was dispersed and ingested by macrophages. Some cells of the ciliary body appeared large and balloon-like with prominent nuclei and nucleoli (Fig. 3). In some specimens, there were large, multinucleated cells resembling sarcomatous changes (noted in previous experiments).  

The lens was cataractous in most cases ranging from slight posterior migration of nuclei to frank Morgagnian globule formation. In some cases the posterior chamber also contained inflammatory cells.

The pars plana, retina, choroid, sclera, and optic nerve in all cases remained free of infiltration (or transformation) (Fig. 6). The retina was detached artificially, but there was no subretinal exudate or retinal involvement. The vitreous appeared to be condensed in some specimens but there was no vitritis.

High levels of virus were found in samples taken from the aqueous humor at the time of enucleation. The amounts of virus in aqueous humor exceeded those in the sera in samples taken at the time of enucleation. FeLV and FeSV were isolated separately and $2.5 \times 10^5 \text{FeLV/ml}$ were found in samples of sera, while $3 \times 10^3 \text{FeLV/ml}$ were found in the aqueous humor. No FeSV was detected in samples of sera while $1 \times 10^2 \text{FeSV/ml}$ were found in the aqueous humor.

In the second group of animals, four out of seven developed a similar clinical histopathologic picture. None of the control animals developed any anterior uveitis. The lower percentage of animals responding in this manner and the somewhat milder inflammatory picture may have been due to loss of virulence or titer of virus accompanying the freeze-thaw process.

At necropsy, all inoculated cats were found to have fibrosarcomas some of which had metastasized to distant sites. One of the animals exhibited a particularly delayed onset of uveitis and was found at necropsy to have no macroscopic fibrosarcomas but many small regressing tumors seen histopathologically.

**Discussion**

Several models of virally induced anterior uveitis have been studied but as yet no consistently pre-
dictable animal model has been proposed to study the mechanisms of production of this type of anterior uveitis. The production of anterior uveitis in the Herpes\textsuperscript{15} and CAV-1\textsuperscript{11} models varies from 20–100\%. Also, development of a fatal encephalitis limits the experimental use of some models.\textsuperscript{14,15}

The advantages of the current experimental system are: (1) the consistent production of an anterior uveitis after subcutaneous injection of a viral agent at a distant site; (2) the ocular structures are left undisturbed by the inoculation procedure and mild uveitis from the procedure alone is therefore avoided; (3) the lack of necessity to use intravenous injection means the immune system may not be as immediately overwhelmed through presentation of an antigen overload to the spleen; and (4) the inflammatory reaction seems to be specific to uveal tissue (more specifically ciliary body) as inflammation was not encountered in other organs studied histopathologically.

We cannot determine from these experiments why this inoculation regime gives a preferential effect for the cells of the ciliary body. We know that in irritative lesions of the anterior segment, the normal functional architecture of the ciliary epithelium is destroyed and

The aqueous humor approaches the composition of plasma. Certainly our histopathologic sections confirm destruction of ciliary processes at the light microscopic level.

The transformation of cells of the ciliary body and presence of virus in aqueous is compatible with the concept that there is preferential viral replication in the ciliary body, with accumulation of virus in the aqueous and perhaps without an efficient means of escape. Perhaps the virus is being actively secreted into the aqueous by the ciliary epithelium and is preferentially proliferating within the eye.

In the model described by Carmichael\textsuperscript{12,13} subcutaneous and intramuscular injection of infectious canine hepatitis virus (ICH) caused an anterior uveitis, centered around the ciliary body and blood vessels of the iris (as well as interstitial keratitis). Intense specific fluorescence was found against viral antigen in the vascular endothelium of the iris, trabecular meshwork, and in macrophages. However, virus could not be recovered directly from serum or aqueous at the time of intense uveitis and corneal opacity formation. Carmichael\textsuperscript{12} concluded that this resulted from the formation of neutralizing antibody that blocked re-

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Fig. 4. Area of chamber angle showing disrupted iris pigment epithelium, large dilated iris vessels, and inflammatory debris in the anterior chamber. The ciliary epithelium is disrupted with fibroblastic proliferation (hematoxylin-eosin, \texttimes 100).
Fig. 5. Area of ciliary body of infected animal, showing intense inflammation with lymphocytes in the outer layers. Large, bizarre cells can be seen in the middle and inner layers (hematoxylin-eosin, X100).

covery of virus. He also concluded that in the case of the ICH model, anterior uveitis was the result of a local hypersensitivity reaction, an Arthus-type reaction as described by Woods. Anterior uveitis appeared only in the presence of both antigen and antibody, but not when virus alone was present in the anterior chamber. This type of reaction is also believed to account for the pathogenesis of other viral anterior uveitides that occur as complications of herpes simplex and zoster infections, smallpox, mumps, influenza, and some viral encephalitides. This would explain why virus cannot necessarily be recovered from the involved tissues. Under appropriate conditions, locally produced antibody, apparently reacts with viral antigen and precipitates on ocular structures. If the inoculum is low, ocularly produced antibody could restrict viral growth and replication. If, on the other hand, virus is inoculated directly into the eye as seen in the lymphocytic chronic meningitis model (and the melanoma model of Neiderkorn et al.) unrestricted viral growth would overwhelm the antibody forming mechanism before it could become functional.

In our model the subcutaneous administration of virus presumably provides a slow, steady presentation of antigen to the spleen and lymph nodes, favoring
the production of a delayed-type hypersensitivity reaction. Once the animal is sensitized to the antigen, \(^5\) liberation of virus into the anterior chamber may act as a challenging dose, inducing the characteristic features of delayed-type hypersensitivity. This situation would mimic immunization of the animal and subsequent challenge through the anterior chamber.

Why this particular inoculation procedure should selectively produce anterior uveitis is unclear. The virus preparation given in the first two inoculations, (MuSV(FeLV)), provides ample sensitization to the virion envelope antigens of FeLV or FeSV (FeLV), but no source of persistent virus because the MuSV genome should not replicate efficiently in cat cells. However, the challenge virus, FeSV-FeLV), does replicate efficiently in nonimmunized cats. Previous studies where the same doses of FeSV(FeLV) were given to cats under the same circumstances resulted in a similar rate of fibrosarcomas but no gross clinical cases of uveitis. Thus, the minimal immune stimulation induced by the FeLV envelope antigens might be an important component in this particular disease process.

We have concluded that the anterior uveitis produced in these animals with this inoculation procedure is most consistent with an inflammatory re-

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**Fig. 6.** Photomicrograph of posterior pole of an eye with intense anterior uveitis. The retina, choroid and optic nerve are normal. The vitreous is clear (hematoxylin-eosin, X40).
sponse generated by the presence of either antibody and virus or some cell-mediated immune response and not due to the infection process because retroviruses, including the ones used in the study, have never been shown to have any cytopathic effect on cells in vivo or in vitro. These viruses are characteristic for their noncytocidal and noncytopathic nature. Also, free antibody has never been found in cats when infectious virus was also present presumably because of the antigen excess. Therefore, free antibody assay in the aqueous humor was not performed.

The relative state of immunologic privilege of the anterior chamber has been studied in a series of experiments by Kaplan and Streilein19-20 and by Kaplan and Stevens.22 They found the afferent and efferent limbs of the immunologic reflex arc intact, but that the lack of lymphatic drainage from the eye results in the absence of lymphocytes from the anterior chamber. However, once the suppressor T-cells are outnumbered by sensitized cytotoxic T-cells and helper T-cells, immunologically mediated tissue destruction may occur.23 Inflammation of this sort should not occur in the animal model for uveal melanoma described by Albert et al.,10 because these animals had not been sensitized previously and, indeed, these eyes are quiet.

The cells of the ciliary body in our experimental animals appear metabolically active possibly malignant or premalignant. It is unlikely, however, that tumor-associated antigen stimulated this inflammatory response, as it is not seen in eyes with fully developed tumors.18 Experiments are now in progress to determine if these cells do become frankly malignant with time.

Key words: uveitis, viral infection, feline sarcoma virus

References