Ocular manifestations of Marek's disease*

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An experimental study of the ocular changes occurring in Marek's disease was carried out. Marek's disease which is induced by a DNA virus, is a disorder that occurs in chickens and related fowl. Ocular involvement is a feature of this disease. In this study the eye changes of Marek's disease were sequentially examined in specific, pathogen-free birds injected with clone-purified Marek's disease virus. An infiltration of malignant-appearing lymphoreticular proliferative cells was seen initially in the optic nerves, ciliary nerves, and uvea in birds receiving the virus either intraocularly or systemically. Subsequent infiltration of similar cells occurred throughout the eye.

Key words: ocular tumor, Marek's disease, avian herpesvirus, DNA virus, lymphoreticular proliferative disease, avian leukosis.

Malignancies of chicken and related fowl are under intensive study because of their significance as a potential model for human cancer. The concept that malignancies can be caused by a virus, proposed by Borrell in 1908, was confirmed in the studies of sarcomas in chickens by Rous in 1911.2 Marek's disease (MD), originally described as a polyneuritis in 1907, has become recognized as an important avian tumor. With advances in cancer virology the focus has shifted from classification according to the tissue involved and tumor cell type to the etiologic agent. The two major types of oncogenic avian viruses are the RNA leukoviruses, a diverse interrelated group, and the DNA herpesvirus, which apparently causes MD. The MD virus was first isolated independently by Churchill and Biggs4 and Solomon and coworkers.5 The MD virus (MDV) has morphologic and immunologic similarities to human herpes viruses.

Eye involvement has been described in naturally occurring forms of malignant diseases of domestic fowl.6-9 These reports, however, do not explain the effect of specific oncogenic viruses on the avian eye, as only presumptive clinical identification of the etiologic agents was made. In a

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This work was supported in part by a grant from the National Institutes of Health, Grant No. 5-R01-CA-6709-10, and by a Public Health Service Grant EY00108-05.

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*Presented in part at the 1973 ARVO meeting in Sarasota, Fla.
recent report, Worthen and Moscovici studied the changes induced in chick embryos by an RNA virus, MC-29.

Methods

Animals. Eight-day-old JM-P line (P-line) chicks, a strain susceptible to MDV, were used for the experiment. As part of a specific, pathogen-free flock maintained at Cornell University, the dams were periodically tested for antibody against avian mycoplasmas and all of the common avian viral pathogens, including MDV and lymphoid leukosis virus (A and B subgroups). Tests conducted before and after chicks were obtained for these experiments were all negative. The progeny carried maternal antibody against the FC-126 strain of turkey herpesvirus which had been used as a vaccine against MD in the dams, but such antibody did not protect chicks against MDV inoculation in other experiments and, therefore, it was not considered a significant factor in this experiment.

Virus. The virus used for inoculation was "clone-purified" MDV, GA isolate. The inoculum consisted of a freeze-dried virus prepared in the manner previously described from a skin extract (enveloped virus from the feather follicle epithelium). The virus was assayed in chicken kidney cell culture and it was found to contain 4,500 focus forming units (FFU) per milliliter.

Experimental design. Birds were infected by two routes and compared to control animals. (1) In 25 birds the virus was injected intra-abdominally (0.1 ml. or 450 FFU per injection) and are referred to as the systemic inoculation group; (2) in 12 birds, intracocular inoculation was used with the virus injected into the pars plana of the left eye (about 0.035 ml. per inoculation), and termed eye inoculation group; and (3) a group of eight birds was kept uninoculated as control animals. The systemic and eye inoculation groups were kept together in one deck of a wire-floored battery brooder in an isolation unit. This facility is designed and managed so as to preclude the likelihood of adventitious infection of avian pathogens. Each unit has an individual air supply and there is an air-lock entrance cubicle where boots, outer garments, and caps are donned. A sink permits washing before entering. The control animals were kept in isolation at another facility.

One to four birds from each of the three groups were killed and examined at 11, 18, 25, 32, 39, and 46 days after inoculation. Studies included gross and histologic examinations of the eyes and viscera; fluorescent antibody (FA) tests for MDV antigen with skin, bursa of Fabricius, kidney, and thymus; and electron microscopic studies (which will be subsequently reported). Eyes were fixed in Zenker's fixative or gluteraldehyde for morphologic study.

Results

A. Birds receiving systemic injection. Chickens killed eleven days after intra-abdominal injection of MDV showed no gross or microscopic systemic lesions. FA tests for MDV antigen were negative on skin, bursa of Fabricius, kidney, and thymus. In the eyes of one of the four birds killed, however, there was a thickening of the arachnoid layer of the optic nerve due to accumulation of lymphoreticular proliferative cells (LPC's) (Fig. 1, A). Similar cells were seen infiltrating a posterior ciliary nerve. There were no other ocular abnormalities seen.

All four chickens killed 18 days after inoculation showed both systemic and ocular involvement. Degenerative changes in the bursa of Fabricius were apparent on microscopic examination. In addition, occasional accumulations of LPC's were seen in the sciatic nerve plexus. Viral antigen (FA test) was detected in all four animals and corresponded quantitatively with the severity of the lesions. This relationship was generally constant throughout the experiment. The most marked involvement was seen in the arachnoid layer with the optic nerve itself also involved. The posterior ciliary nerves also showed promi-
inent infiltration. Layers of LPC's, five to six cells deep, were present on the anterior surface of the irides of two animals. In another pair of eyes there was a small focus of LPC's at the point of attachment of the pecten to the optic nerve, as well as a considerable infiltration of tumor cells in the corneal stroma. The two control chickens had no abnormalities.

Animals killed 25 days after inoculation had more extensive lesions. On histopathologic examination slight degenerative changes were seen in the bursae of Fabricius of all four birds, and in two birds there was evidence of active repair. Moderate numbers of LPC's were observed in the brachial plexus, sciatic plexus, and spleen of all four animals. Tests for FA antigen were strongly positive in all four birds. Gross systemic lesions, however, were seen in only two birds. These consisted of moderate to severe diffuse tumor involvement in the kidney, proventriculus, liver, gonad, and the bursa of Fabricius. The ocular lesions were severe. In all four fowl the choroid and ciliary body had diffuse infiltrations of LPC's with the presence in some areas of larger focal accumulations (Fig. 1, B and C). In one bird there were LPC's between the scleral lamellae. The ciliary nerves were involved (Fig. 2, A).

In one pair of eyes, LPC's were present in the conjunctival stroma at the limbus. The corneal endothelium appeared necrotic (Fig. 2, B) and the corneal stroma showed increased cellularity. The pecten of all specimens contained numerous LPC's at its point of attachment, but the interior of the pecten looked quite unremarkable. The two control chickens were unremarkable.

All four birds killed at 32 days had grossly observable systemic lesions, two mild involvement, and two quite extensive. FA antigen was detected in all four chickens. These birds had fewer infiltrative changes in the gonad, brachial and sciatic plexuses, and liver than had been observed in the animals killed a week previously. The involvement of the bursa of Fabricius and spleen with LPC's was similar to that in the 25-day group. In the eyes of three of the four birds there was similar but less severe involvement in the uvea and optic nerve than had been noted the previous week. Involvement of the ciliary nerves was
again seen. In one of these birds' lens cell nuclei persisted to the center of the lens (Fig. 2, C). In the remaining animal there were large masses of LPC's present in the optic nerve which exceeded in size any of the lesions previously seen. The two control birds were unremarkable.

At 39 days there were marked gross lesions of viscera but their histopathology and the presence of FA antigen were not studied. In the eyes of two of the chickens there was little difference from those examined the previous week. The severity and distribution of the LPC's generally corresponded to that seen in the three animals with moderate involvement at 32 days. There did appear to be a more marked involvement in the pecten, however. The two control chickens were normal.

In all 4 birds examined at 46 days there was very little involvement in the choroid, but the stroma of the iris and ciliary body appeared replaced by massive numbers of LPC's. The optic nerve and meninges were about the same as described previously. The cells of the corneal epithelium had an atypical appearance in areas and resembled LPC's. This change was not seen in the animals studied earlier. The control birds again had no lesions.

B. Birds receiving ocular injection. The pattern of ocular involvement was similar in the systemic and intraocularly inoculated groups (Table 1). The major differences in the eye lesions between systemically and intraocularly inoculated birds were that: (1) some variability was seen in the onset of the ocular changes (Table 1); and (2) the degree of ocular involvement was greater in the intraocular injected group. An additional unusual finding was seen in one chicken killed 11 days after receiving
Fig. 3, A. Lens, P-O section, 11 days after intraocular injection. (Hematoxylin-eosin, x400.)

Fig. 3, B. Lens, iris, ciliary body, and cornea, P-O section, 25 days after intraocular injection. (Hematoxylin-eosin, x50.)

an ocular injection of MDV: the lens epithelium showed proliferation of atypical appearing cells with prominent mitotic figures (Fig. 3, A).

Significantly, except for atypical lens epithelial cells the left (injected) and the right (uninjected) eyes showed similar changes. Systemic involvement was the same in its course and severity in the intraocularly and parenterally injected groups.

The marked ocular involvement in this group of birds is particularly well illustrated in the eyes of one bird killed at 25 days after intraocular inoculation (Figs. 3, B and C): the arachnoid layer was thickened by LPC's and there was a diffuse infiltration of these cells in the pecten and optic nerve. The choroid contained scattered LPC's while the normal structure of the ciliary body and iris were replaced by massive tumors composed of LPC's, many with mitotic figures. The anterior chamber also contained similar cells and proteinaceous material. In the two birds studied at 39 days there was prominent involvement in the optic nerve (Fig. 4, A).

At 46 days the corneal stroma was more cellular than in the control animals (Fig. 4, B) and there was foci of LPC's in the corneal epithelium (Fig. 4, C). These changes were nearly identical to what was previously described for the systemically injected birds.

C. Spontaneously occurring disease. The eyes of four adult chickens with severe involvement of MD, diagnosed on the clinical bases of paralysis and impaired vision, were examined. There were LPC's in the cornea, iris, ciliary body, choroid, optic nerve, ciliary nerves, and pecten. The sites of involvement and the type of infiltrating cells were identical to that found in our inoculated study. In additional, in these birds atypical proliferating cells in the lens were consistently seen identical to those shown in Figs. 3, A and B.

Discussion

A. Significance of present experiments.

By using purified MDV and specific, pathogen-free birds this work provides, for the first time, the sequential ocular changes occurring in MD. It appears likely that the
eye picture of MD is relatively specific and may be useful in differentiating MD infection from RNA virus-induced tumor diseases in chickens. This provides an in vivo model of neoplastic ocular disease resulting from infection with an oncogenic DNA virus.

The changes occurring in the cornea and lens are of particular interest and may be the result of local effect of the virus on these tissues. It is probably not related to a genetic factor because lens changes are readily apparent in most cases of spontaneous disease with many strains. Malignant transformation of the lens epithelium has not been reported clinically in any species although it has been described in previous experiments. The observation of retained nuclei at the center of the lens has previously been described in rubella but its finding in this disease suggests that other viruses may cause this abnormality.

In the P-line of chickens employed in this study, it appears that the GA strain is sufficient to induce full-blown tumors without need for additional RNA virus as implicated in other lines (LSI-SPF) as recently reported.18

B. Historical review of MD. Ocular involvement in MD is considered a primary characteristic and has been variously referred to as “pearly eye,” “gray eye,” or from an histopathologic standpoint “ocular lymphomatosis” or “ocular leukosis.”

Since Marek's original description in 1907, a massive literature on this disease has accumulated and reviews are available.4 An extensive review of the history of the ocular findings has also been prepared.19 Most of our knowledge of MD is based on clinical and pathologic studies and it should be noted that most avian pathologists still make their diagnosis on these criteria rather than isolation of virus and identification of antibodies.

Between 1929 and 1943, a number of descriptions of the ocular changes occurring in presumed MD were published. According to these descriptions, the most prominent change was iris involvement, but descriptions of the histopathologic changes particularly as regarding the type of cells infiltrating the iris varied considerably. A comprehensive review of the clinical and
pathologic eye changes is that by Nelson and Thorp. As in all of these descriptions, considerable controversy still exists as to whether the eye changes are inflammatory in nature or neoplastic.

The authors wish to thank Mrs. Indu Bhatt, Miss Chris Hogan, Miss Jan Wilcox, Miss Susan Smith, and Mr. Raymond Harris for their technical assistance in the preparation of the histologic materials. The secretarial help that Mrs. Louise Redfield has given us is also appreciated.

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