Marek's disease virus infection in chickens: A natural model for VZV-induced eye lesions

A.R. Pandiri, A.L. Cortes, I.M. Gimeno

Department of Population Health and Pathobiology

College of Veterinary Medicine, Raleigh, NC 27606

NC STATE UNIVERSITY

Abstract

Marek's disease virus (MDV) is an alpha-herpesvirus of chickens that shares numerous similarities with varicella-zoster virus (VZV). These viruses have similar genomic organization, viral biology and induce similar pathology in their respective hosts. As VZV, MDV is able to induce several lesions in the eye including keratitis, uveitis, and retinal necrosis. The main objective of this work was to further characterize MDV infection in the eye and to establish temporal relationship of viral infection to development of eye lesions. The early lesions consisted of macrophage infiltration within the choroids (4 dpi) followed by vasculitis and perivascular infiltration of lymphocytes, plasma cells and granulocytes (11 dpi). Expression of early MDV antigen pp38 in the cells infiltrating choroids was first detected at 11 dpi. By 26 dpi, multifocally the retinal pigmented epithelium (RPE) and photoreceptors were destroyed along the peripheral retina. In addition, degenerative changes were observed in the inner nuclear layer and abundant MDV viral antigen was found in all layers of the retina. The retinal architecture was completely effaced by 56 dpi. The RPE and photoreceptors were no longer detectable. There were extensive areas of necrosis in the inner nuclear, inner plexiform and ganglion cell layers. We propose that MDV infection in chickens is a natural model to study the pathogenesis of VZV-induced eye lesions. The current experimental models involve intraocular inoculation of human viruses into various rodents. The unnatural route of inoculation and lack of host-pathogen coevolution may not reflect the actual disease process. The advantage of this model is that chicken is the natural host for MDV and we can study the early stages of infection without intraocular inoculation.

Introduction

Marek's disease virus (MDV), an oncogenic lymphotropic Alphaherpesvirus, induces lymphomas, immunosuppression and various neurological syndromes. The ocular tropism of MDV was demonstrated even before the identification of the virus. However, the histologic nature of all the previously recognized and described ocular lesions was ambiguous, i.e. neoplastic *vs.* inflammatory (Smith *et al*., 1974, Ficken *et al.,* 1991). We have recently identified that the eye lesions can occur very early after MD infection and are primarily inflammatory in nature (Gimeno and Silva, 2006).

Ocular lesions induced by human herpesviruses include keratitis, keratoconjunctivitis, iridocyclitis, chorioretinitis, retinal necrosis, vitreitis, and optic neuritis. Currently, intraocular inoculation of human herpesviruses into various rodents and non-human primates is used to study the pathogenesis of human herpesvirus-induced ocular disease. Some of the models include examination of the uninoculated contralateral eye following uniocular anterior chamber inoculation of herpes simplex virus (HSV)-1 in BALB/c mice; supraciliary inoculation of murine cytomegalovirus in euthymic mice; intrastromal inoculation of Varicella-Zoster virus (VZV) in a Guinea pig; intrastromal and subconjunctival injection of Simian Varicella Virus in African green monkeys (Whittum *et al*., 1984, Atherton *et al.,* 1991, Pavan-Langston *et al*., 1989, Metcalf *et al.,* 1995).

MDV also induces a spectrum of ocular lesions that are very similar to the human herpesvirus-induced ocular lesions. In addition, MDV and VZV share several biological and genetic features (Osterreider *et al.,* 2006). Due to the similar ocular lesions and biological and genetic similarity between the VZV and MDV, we propose that MDV infection in chickens could serve as a valuable model to study the pathogenesis and immunology of the VZV-induced ocular lesions.

Objective

Characterization of MDV-induced ocular lesions and establishment of temporal relationship between viral infection and development of ocular lesions

Materials and methods

Experiment #1: Forty four highly susceptible laboratory strain of chickens (15x7) were inoculated at day of hatch with a virulent MDV strain Md5. Seventeen negative controls were inoculated with tissue culture medium. Chickens were euthanatized and sampled on 4, 6, 8, 10, 11, 26 and 56 days post inoculation (dpi).

Materials and methods (Contd..)

Experiment #2: Twenty SPAFAS chickens were inoculated with a very virulent plus (VV+) MDV strain 648A. Eight negative control chickens were inoculated with tissue culture medium. Chickens were euthanatized and sampled at 11, 18, 26 and 31 dpi.

At necropsy, tissues were fixed in 10% neutral buffered formalin or Bouin's fixative for routine hematoxilin and eosin staining and were also embedded in Tissue-Tek® O.C.T compound (Sakura Finetek USA, Inc. Torrance, CA) and snap frozen in liquid nitrogen for immunohistochemistry studies.

Immunohistochemistry. All tissues were stained with a panel of immunologic markers (CT4 (CD4), CT8 (CD8), M4 (IgM), Cla (MHC II), KUL01 (Macrophages) (Southern Biotech, Birmingham, Al) and virologic markers (H19 (pp38- early viral antigen, Cui *et al.,* 1990), 1AN86.17 (gB - late viral antigen, Cui *et al.,* 1990) and 23B46 (meq – oncogene, Liu *et al.,* 1996)). A modified avidin-biotin-peroxidase complex method (Hsu *et al*., 1981) using the Vectastain® ABC kit (Vector Laboratories, Burlingame, CA) was performed on 5 µM cryosections. Specific brown DAB or Vector Red (Vector laboratories, Burlingame, CA) staining with hematoxylin counter stain was used.

Results

Histological lesions (Fig. 2, and Table 1): The histologic changes within the eyes were classified into early (≤ 11 dpi) and late lesions (≥ 18 dpi).

The earliest (4 dpi) ocular lesions occured within the choroid layer. The vascular endothelial cells were hypertrophied, and there was mild perivascular infiltration of macrophages and lymphocytes. By 11 dpi, there was moderate infiltration of lymphocytes, plasma cells, macrophages and occasional heterophils within the choroids, ciliary body and iris.

On 18 dpi and later, the iris, ciliary body and choroids were markedly expanded by lymphocytes, macrophages and plasma cells. In addition, the optic nerve, pecten, vitreous, and retina (especially peripheral retina) were expanded by increasing infiltration of lymphocytes, plasma cells and macrophages. The vitreous cell infiltrate initially invaded and effaced the ganglion cell layer and inner nuclear layer, and later the choroid cell infiltrate invaded the retinal pigment epithelium as well as the outer retinal layers. Eventually, the choroid and vitreous cell infiltrate diffusely effaced all the retinal layers or caused segmental to diffuse retinal detachment. There was mild parakeratosis with occasional multifocal disruption, swelling and vacuolation of the corneal epithelial cells. The corneal stromal tissue was diffusely edematous with moderate infiltration of lymphocytes, plasma cells and macrophages as well as multifocal neovascularization.

Immunophenotypic characterization (Fig. 3,4 and Table 1): Within the early lesions, the cellular infiltrates within the choroids, ciliary body and iris stained strongly positive for CD8 and MHC II, and mildly positive for macrophages and CD4. However, within the late lesions, the infiltrating cells within the uveal tract, pecten and retina were mildly positive for CD4, CD8, and macrophages but the cells remained strongly positive for MHC II.

The infiltrating cells within the early lesions were negative for gB, and *meq* but scattered cells were positive for pp38 antigen within the choroids. Within the late lesions, the infiltrating cells were negative for gB and *meq* but positive for pp38 antigen within the uveal tract, retina, and cornea.

Figure 1: The normal eye on the left had sharp margins of the pupil where as within the affected eye, the pupil margins were irregular and the cornea is cloudy with off-white opacity accompanied by congested sclera and conjunctiva.

Results (Contd..)

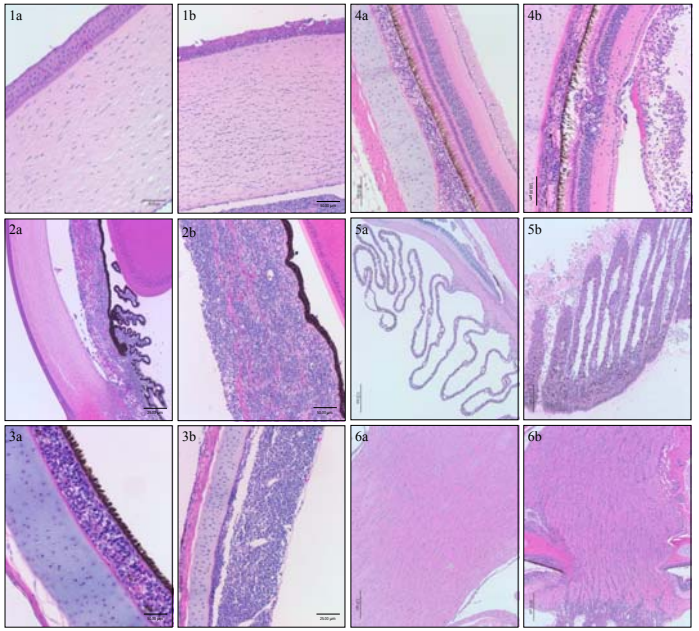

Figure 2: Photomicrographs of H&E stained histologic sections labeled 1a-6a and 1b-6b indicate early and late lesions, respectively, within the eyes of chickens infected with MDV. 1a&b - cornea, 2a&b – iris, 3a&b – choroids, 4a&b – retina, 5a&b – pecten and vitreous, and 6a&b – optic nerve. The early lesions were limited to uveal tract whereas the late lesions extensively affected the uveal tract, pecten, vitreous, retina and optic nerve.

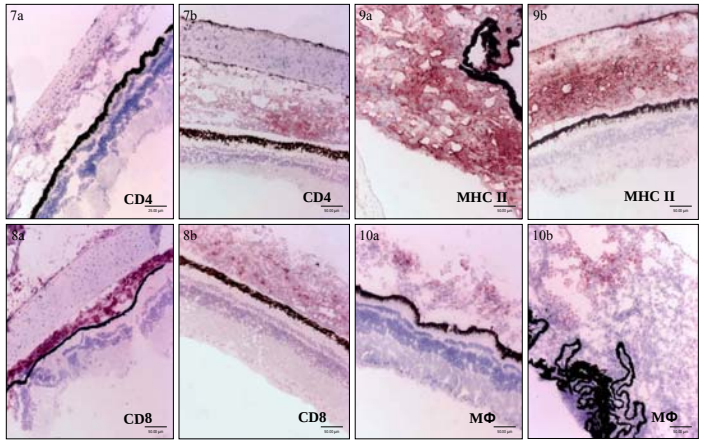

Figure 3: Photomicrographs of cryosections with immunohistochemical detection of CD4 (7a&b), CD8 (8a&b), MHC II (9a&b) and MΦ (10a&b). The sections labeled 7a-10a and 7b-10b indicate early and late lesions, respectively, within the eyes of chickens infected with MDV. Vector Red chromogen with light hematoxylin counterstain, Avidin-biotin-peroxidase (ABP) complex method.

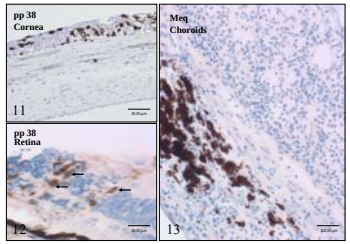

Figure 4: Photomicrographs of cryosections with immunohistochemical detection of pp38 within the corneal epithelium (11) and retina (12). Meq antigen expression was not found in all the sections examined. Retina (13) with dispersed retinal pigment but no meq antigen expression. DAB chromogen with light hematoxylin counterstain, ABP complex method

Table 1. Summary of MD-induced ocular lesions, immunophenotype and viral antigen expression of the infiltrating cell population

Characteristics	Early lesions (up to11 dpi)	Late lesions (18 dpi on)
Location	Uveal track	Uveal track, Cornea, Retina, Pecten and Vitreous
Cell phenotype infiltrates	CD4+, CD8++, MΦ+, MHC II ++	CD4+, CD8+, MΦ+, MHC II ++
Viral antigen expression	pp38+, gB-, meq -	pp38++, gB-, meq-

Conclusions and discussion

The results of this study indicated that MDV infection in chickens induces severe ocular lesions. These lesions could be categorized into early and late based on the extent of infiltration, distribution, immunophenotype of the infiltrating cell population as well as the viral antigen expression. The early lesions were characterized by moderate lymphohistiocytic uveitis and the immunophenotype of the infiltrating cell population indicated strong CD8 and MHC II expression and mild CD4 and macrophage antigen expression. Within the early lesions, expression of viral antigen pp38 was limited to the choroids. The late lesions were characterized by severe lymphohistiocytic uveitis, keratitis, pectenitis, vitreitis, optic neuritis, retinitis and segmental to diffuse retinal necrosis. Within the late lesions, expression of pp38 was seen within the uveal tract, cornea, and retina. Surprisingly, meq antigen expression was not detected and CD4 expression within the infiltrating cells was low, in contrast to antigen expression and immunophenotype of the MD lymphomas. Based on the above features, the ocular lesions seem to be inflammatory rather than neoplastic in nature.

MD-induced ocular lesions recapitulate several features of VZV- and HSV-induced ocular disease. In addition, MDV shares close biologic and genetic similarity especially with VZV, another alphaherpes virus. MD is very well characterized and there are numerous well characterized viral and vaccine strains as well as chicken strains to study the spectrum of ocular lesions. The only drawback might be the avascular retina of the chicken. However, pecten appears to substitute well for the avascular retina and the chicken eye have served as useful model to study various degenerative disorders in humans. The current rodent models were helpful in providing some information on the probable pathogenesis of HSV- and VZV-induced ocular lesions. However, the current experimental models involve intraocular inoculation of human viruses into various rodents. The unnatural route of inoculation and lack of host-pathogen coevolution may not reflect the actual disease process. The advantage of MD model is that chicken is the natural host for MDV and we can study the early stages of infection as well as immune responses without intraocular inoculation.

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