Simian virus 40-induced retinopathy in the rat*

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Neonatal albino rats received Simian virus 40 (SV40) within 24 hours of birth. Twenty-four to 48 hours later viral antigen was demonstrable within the retina by immunofluorescent studies. Histopathologic examination of the retinas five and ten days after infection revealed basophilic intranuclear inclusions in cells of the inner nuclear layer. Adult rats similarly infected as newborns developed ophthalmoscopically visible neovascular tufts on the retinal surface by the sixth month of neonatal life. Histopathologic study of the affected adult rat eye revealed preretinal and subretinal neovascularization, retinal folds, and gliosis. Many of the features of SV40-induced retinopathy in the rat are similar to those seen in lymphocytic choriomeningitis virus-induced retinopathy in the rat and sheep blue-tongue virus-induced retinal dysplasia in the fetal lamb.

Key words: Simian virus 40, retinopathy, retinal neovascularization, retinal fold, immunofluorescent, fluorescein angiography.

There has been an increasing interest in the relation between viruses and degenerative diseases of the eye. Several viruses, such as the virus of subacute sclerosing panencephalitis, rubella virus, cytomegalovirus, and herpesvirus, have been associated with ocular disease in the human. Recently, Silverstein and co-worker, in two separate studies have produced viral-induced retinopathies in animal models utilizing the sheep blue-tongue virus and lymphocytic choriomeningitis virus.

Albino rats, infected as newborns with Simian virus 40 (SV40) as part of an oncogenic study, were noted to have developed ocular abnormalities. This paper is concerned with our investigation of these abnormalities.

Materials and methods

Study 1. Infected and control albino rats obtained from the oncogenic study were examined ophthalmoscopically and fluorescein angiography was performed on several of the rats displaying retinal abnormalities. The animals had been infected within 24 hours of birth with 0.1 or 0.2 ml. of live SV40 by intravenous or intraperitoneal administration. Both affected and non-affected as well as control rats were killed and their eyes...
Fig. 1. Fluorescein retinal angiogram of adult rat 5 months following infection with SV40 showing: filling of neovascular tufts on retinal surface and marked leakage of dye.

processed for histopathologic examination.

Study 2. Newborn albino rats were infected with live SV40.* The animals received 0.2 ml. of undiluted live virus either intravenously or intraperitoneally. Infected animals and paired control animals were killed at the following postinfective intervals: 1, 2, 5, and 10 days. The eyes were enucleated and either fixed in 10 per cent neutral-buffered formalin or quenched in liquid nitrogen.

For light microscopy, formalin-fixed eyes were embedded in paraffin, sectioned at 9 microns, and stained with hematoxylin and eosin. For immunofluorescent studies, cryostat sections were cut at 8 microns and allowed to air dry prior to staining. Fluorescent antibody staining utilized the indirect technique by first treating the sections with goat anti-SV40 globulin† and then with fluorescein conjugated rabbit anti-goat globulin.‡ Appropriate control sections were treated first with goat globulin and then with fluorescein-conjugated rabbit antigoat globulin. The sections were viewed under ultraviolet light at 365 nm.

Study 3. Adult albino rats received an intraperitoneal injection of 0.2 ml. SV40. Infected and paired control animals were killed at 1, 5, and 10 days after infection. Eyes were prepared and studied as delineated in Study 2.

Results

Study 1. Adult rats from the oncogenic study had ophtalmoscopically visible neo-

vascular tufts on the retinal surface. Fluorescein angiography demonstrated filling of the neovascular tufts in the arterial phase with subsequent leakage of the dye (Fig. 1).

Histopathologic study of the retina of adult rats from the oncogenic study showed several characteristic abnormalities, and gliosis in the outer retina with neovascularization, "rosette" formation, absence of photoreceptors. The neovascularization occurred as either a preretinal (Fig. 2A) or a subretinal type (Fig. 3). On occasion, the preretinal growths were extensive and were associated with retinal detachment, cataract formation, and cyclitic membrane. The

Fig. 2. Adult rat 6 months following infection with SV40: A. Low-power photomicrograph showing extensive retinitis proliferans and disorganization of the outer retina with formation of rosette in area of subretinal neovascularization. Note posterior migration of subcapsular lens epithelium. (Hematoxylin and eosin, x125.) B. Higher power view showing the rosette formation due to infolding of the retina in areas of subretinal neovascularization. The course of the vessel downgrowth is delineated by the arrows. (Hematoxylin and eosin, x320.)
subretinal neovascular growths were from vessels in the superficial retina (Fig. 2B) and in some cases displayed a large fibrous component (Fig. 3). The formation of retinal “rosettes” appeared in areas of neovascularization secondary to foldings of the retina (Fig. 2B). Large retinal folds were also noted in conjunction with neovascular proliferation (Fig. 4). The retinæ in these eyes showed extensive loss of photoreceptors with marked gliosis in the outer retina (Figs. 2B and 3). These findings were always in relation to the degree of neovascularization and “rosette” formation. In those areas of the retina in which neovascularization was absent, normal retinal anatomy was preserved. The retinal pigment epithelium and choroid appeared uninvolved. A general postmortem examination of animals in this group was otherwise unremarkable.

**Study 2.** Twenty-four to 48 hours following intravenous or intraperitoneal administration of SV40 to the neonates, fluorescent-antibody staining demonstrated viral antigen within inner retinal and pigment epithelial cells (Fig. 5). Uninfected control animals were negative.

The 5-day-old intravenously or intraperitoneally infected neonatal rat retina showed separation of the neuroblastic layer into the future inner and outer nuclear layers. At this time, the nuclei of cells in the inner portion of the neuroblastic layer showed densely basophilic intranuclear inclusions. The cells in the outer portion of the neuroblastic layer showed formation of large, round, pale-staining granular nuclei.

The 10-day-old, intravenously or intraperitoneally infected rat retina showed complete separation into inner and outer nuclear layers. Many of the cells of the inner nuclear layer had densely basophilic intranuclear inclusions, and the vessels in the inner retina displayed prominent endothelial proliferation (Fig. 6).

**Study 3.** Adult rats infected with SV40 did not demonstrate histologic lesions compatible with infection or its sequelae.

**Discussion**

SV40 is one of the oncogenic DNA viruses belonging to the papova group. Following infection, SV40 may exert either of two cellular effects: productive infection with lysis of permissive cells or transformation. SV40 has been shown to transform astrocytes in vitro and to produce tumors in hamsters and rats. Recently, a virus identical to or closely associated with SV40 has been isolated from the brains of patients with progressive multifocal leukoencephalopathy (PML). In PML, virus-like inclusions have been observed within the nuclei of glial cells and electron microscopy has demonstrated the presence of virus-like particles within...
Fig. 5. Immunofluorescent staining of neonatal rat retina 24 hours after infection with SV40. Viral antigen is demonstrable in the inner retinal layers (i) and in the retinal pigment epithelium (r) (coronal section). ×320.

Fig. 6. The rat retina 10 days after infection with SV40. Many of the nuclei in the inner nuclear layer show densely basophilic intranuclear inclusions. Vessels in the inner retina show prominent endothelial proliferation (e). These inclusion bodies.14 Black and Hirsch15 have suggested that, if PML is due to SV40, it may represent a transformation of the astrocytes by the virus.

In the present study, neonatal rats were infected with SV40 within 24 hours of birth. Viral antigen was demonstrable in the retina within 48 hours and was confined to the inner retinal layer and the retinal pigment epithelium. Histopathologic changes were first apparent within 5 days after administration of the virus and were characterized by the appearance of apparent viral inclusion bodies at the inner portion of the neuroblastic layer. At a later stage, retinal neovascularization with secondary “rosette” formation and degeneration of the outer portion of the retina dominated the histopathologic picture.

Monjan, Silverstein, and Cole5 studied the retinopathy produced by intracerebral inoculation of lymphocytic choriomeningitis virus (LCM) in the newborn rat. They showed viral antigen by immunofluorescent studies at four days of age within the inner portion of the neuroblastic layer and at ten days of age within the inner nuclear layer and in retinal pigment epithelium cells. The demonstration of LCM antigen in these layers is quite similar to the presence of SV40 in our studies. At a later stage in the course of the LCM infection, complete destruction of all retinal elements occurred save the retinal pigment epithelium.

Silverstein and co-workers6 produced retinal dysplasia accompanying an extensive destructive retinopathy and hydrancephaly in the fetal lamb with the attenuated blue-tongue virus. In their study, retinal development progressed normally up to a point where virus-induced destruction of the various retinal elements led to a disorganization and attempt at repair. These mechanisms were only operative when the retina was immature and rela-
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tively undifferentiated. In both the SV40 and sheep blue-tongue virus-induced retinopathies, histopathologic changes are seen in the vessels and in the neuroblastic layer in the early period after infection. The relationship in the SV40-induced retinopathy between histopathologic changes in the retinal vessels and subsequent development of neovascularization is currently under investigation.

Several features, as noted by Silverstein and co-workers, are common to these three models of viral-induced retinopathy. These are: the presence of an immature retina, formation of early histopathologic lesions in the inner retina, and the lack of a significant inflammatory response in the presence of widespread retinal degeneration. A feature of the SV40-induced retinopathy not present in the other animals is the induction of retinal neovascularization. The etiology of this late-developing phenomenon remains obscure but two possible etiologies exist. Retinal neovascularization may develop, as proposed by Wise and others as a result of retinal hypoxia, which in this instance may be related to the SV40-induced lesions. SV40 on the other hand may have exerted a vasoproliferative stimulus on retinal vascular endothelial cells. This problem is currently under investigation.

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