Ocular pathology induced by the suckling mouse cataract agent

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The eye disease caused by SMCA may be best characterized as an endophthalmitis, with early retinitis followed by subsequent posterior uveitis. Pathologic features of lens include: proliferation and abnormal posterior extension of lens epithelium, increased accumulation of lens capsule material, and production of aberrant lens substance. Cataractous change appears to be secondary to intraocular inflammation. In addition to the above pattern, in roughly 20 per cent of all cases, 3 weeks after inoculation, lens capsule is destroyed giving rise to a foreign body granulomatous reaction.

Key words: Suckling mouse cataract agent (SMCA), cataract, mycoplasma, spiroplasma, endophthalmitis, retinitis, lens, ocular pathology.

The suckling mouse cataract agent (SMCA) was isolated between 1960 and 1962 from rabbit ticks, during a search for rickettsia.1 When injected intracerebrally into newborn CFW mice, it produced a strikingly high incidence of cataract formation, usually bilateral, appearing grossly between 15 to 31 days after injection. Subsequent studies, including morphologic data, based on electron microscopic observation, suggested that the agent was actually a previously undescribed form of mycoplasma or mycoplasma-related agent.2 Recently, the SMCA has been cultivated in artificial media and identified to be a member of a class of plant and insect mycoplasma known as "spiroplasma."3

The incidence of cataract formation in certain rat strains is nearly 100 per cent.4 More severe than the condition in mice, the rat eye disease has slightly earlier onset and greater inflammatory change. In addition to cataract formation, the agent in rodents produces chronic encephalitis, hydrocephalus, retarded development wasting, and even death. Disease has been produced only in newly hatched chicks and newborn mice and rats, but not in the adult form of these species.

Olmsted and associates5 examined involved mouse eyes by means of slit lamp technique and also offered a short review of the sequence of histopathologic changes.

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In discussions of the SMCA-induced eye disease in mice and rats, Clark referred briefly to some microscopic features. Zeigel and Clark, by means of electron microscopy, examined infected rat retinas to gain information regarding the morphology and localization of the agent. However, to date, no sufficiently detailed histopathologic investigation of the SMCA-induced eye disease has been reported. It is the purpose of this paper to document the progression of histologic changes associated with the eye infection in the rat, emphasizing the phenomenon of the cataract formation.

Materials and methods

SMCA with a history of 11 to 12 passages was grown in the yolk sac of embryonated chick eggs according to the method described by Clark. Pooled allantoic fluid was back-titrated in eggs, and estimated to have a titer of $10^{-4}$ egg lethal dose-50% (ELD50) per milliliter. Twelve litters of newborn rats of the Sprague-Dawley strain, at less than 72 hours of age, were inoculated in the left cerebral hemisphere with 0.02 ml. of SMCA stock. Four litters of control newborn rats of the same age were inoculated in a similar fashion with an equivalent amount of normal allantoic fluid. For 3 weeks following inoculation, animals were sacrificed daily. Experimental and control animals were sacrificed over the next 17 weeks at intervals varying from every 3 days to once per week. Enucleated eyes, totaling 200 experimental and 60 control, were fixed in 10 per cent neutral formalin, dehydrated in ethyl alcohol, and embedded in glycol methacrylate. Sections cut at 3 \( \mu \)m were stained with hematoxylin-eosin and periodic acid-Schiff reaction, and examined by light microscopy.

Results

The earliest pathologic change occurs on day 3 post injection (p.i.). At this time, mild to moderate infiltrate of polymorphonuclear leukocytes and round cells appears within the vitreous cavity. The inflammatory infiltrate involves the inner layers of the retina. The choroid coat and optic nerve are unaffected.

The earliest lens pathology is present at 5 days p.i., and consists of scattered degeneration and karyorrhexis of the lens epithelial cells. Fine vacuolization of the subepithelial lens substance, commonly present in the lens of newborn rats, appears abnormally increased. Infiltration of the vitreous cavity becomes more profound, and the full thickness of the retina contains inflammatory cells. The cytologic appearance of all retinal layers is disturbed with the exception of the photoreceptor cells. The optic nerve is first involved with acute inflammation at this stage.

By 1 week p.i., lens cells of the bow region fail to undergo normal elongation and maturation (Fig. 1). Many epithelial cells form a cluster at the equator, and the cells which have elongated to form the bow
Fig. 2. Two weeks p.i.: Equator. A, Lens epithelial cells proliferate into multilayers with formation of subjacent swollen nucleated lens cells. (Hematoxylin and Eosin; ×350.) B, Lens capsule thickening and tiny scattered nodules of basement-membrane material are associated with the multilayer pattern of epithelial cells. (Periodic acid-Schiff; ×350.)

configuration fail to denucleate. Also, abundant nuclear debris is scattered within the superficial lens substance. The retina displays increased inflammation with greater disruption of ganglion cells and, for the first time, destruction of photoreceptor cells. The cornea is infiltrated with polymorphonuclear leukocytes and has swollen endothelium. Both the anterior and posterior chambers contain serum and polymorphonuclear leukocytes. The anterior sclera and the ciliary bodies are diffusely infiltrated with inflammatory cells.

At 2 weeks p.i., lens epithelium develops into multilayers two to three cells thick. This change is most pronounced at the equator. Deep to this multilayer pattern is formed aberrant lens substance, composed of large clusters of swollen lens cells, which retain their nuclei (Fig. 2, A). Adjacent to these areas of epithelial proliferation, the lens capsule exhibits diffuse thickening. Also, tiny nodules of basement-membrane material appear scattered through these regions (Fig. 2, B). With development of the multilayer pattern, many epithelial cells at the equator continue to extend posteriorly (Fig. 3). This posterior epithelium forms islands of cells in several cases (Fig. 3). Two weeks p.i.: Equator. Lens epithelial cells extend posteriorly beyond the equator (arrows). Note that these epithelial cells pass beyond a degenerated bow nucleus configuration. (Hematoxylin and Eosin; ×225.)
Fig. 4. Two weeks p.i.: Posterior lens. Islands of lens epithelium (arrows) appear at the posterior aspect of the lens. The central lens substance exhibits central liquefaction necrosis, with a surrounding cortical zone of swollen fibers. (Hematoxylin and Eosin; x80.) Inset: A magnified view of some of the abnormally located epithelial islands. (Hematoxylin and Eosin; x330.)

Fig. 5. Two and one-half weeks p.i.: Posterior lens and retina. Epithelial foci give rise to elongating lens fibers, which resemble bow configurations. Note the associated PAS-positive nodules. Retinal photoreceptor cells undergo metaplasia to form a rosette-like structure. (Periodic acid-Schiff; x130.)

4). Degenerative changes of the central lens substance first become manifest at this point and consist of diffuse, central liquefaction necrosis, with slight vacuolization. An intact cortical zone of swollen, viable lens fibers still remains.

The degree of posterior extension of lens epithelium is much greater by 2½ weeks p.i., with these abnormally located foci of cells developing into the multilayer pattern similar to that noted anteriorly. Some of these posterior epithelial foci give rise to clusters of elongating lens fibers (Fig. 5), resembling bow configurations normally found at the equator region, and are accompanied by scattered nodules of basement-membrane material. The posterior lens capsule is diffusely thickened.
Fig. 6. Three weeks p.i.: Marked degeneration of the central lens substance increases, while
the superficial lens substance exhibits dense staining and calcification. A continuous band
of epithelium, with a multilayer pattern, encircles the lens. (Hematoxylin and Eosin; x43.)

Retinal changes from 2 to 2½ weeks consist of marked focal thinning, due to
disappearance of the outer layers of the retina. Inflammatory cell infiltrate, noted
previously in the retina, has become significantly reduced. Some remaining photo-
ceptor cells undergo metaplasia to form rosette-like structures (Fig. 5). The choroid
coat for the first time has diffuse inflammatory cell infiltrate, but it clears within a
relatively short period.

Islands of epithelium, at the posterior aspect of the lens, become fused to give
rise to a continuous band of epithelium about the entire perimeter of the lens. The
epithelium throughout has undergone a more pronounced proliferation into a multi-
ple-cell layer pattern. The degenerative change of the central lens substance be-
comes more extensive. The surrounding thin zone of superficial lens fibers is deeply
eosinophilic with deposition of calcium (Fig. 6).

In roughly 20 per cent of all cases, after 3 weeks, liquefaction necrosis of the lens
may be so great as to result in breakage of the capsule, usually at its posterior aspect.
The coiled lens capsule remnants are engulfed by phagocytic, multinucleate, giant
cells. Thick granulomatous tissue, consisting of inflammatory cells, multinucleate
giant cells, and patchy necrosis, replaces the retina and choroid coat. In those cases
with lens destruction, by 5 weeks p.i., lens substance is totally resorbed, and there re-
mains a low-grade, smoldering, foreign-body reaction to lens capsule fragments.

In the majority of cases, namely those which have no capsule breakage, the cata-
ract reaches its end stage by the fourth week p.i. The substance of the lens is
markedly shrunken, ovoid in shape, with resultant folding of the redundant capsule
(Fig. 7). From the fourth week p.i. on, the only significant change noted is a steady
lamellar thickening of the capsule, plus an increase in the number and size of base-
ment-membrane nodules, many of which are located subcapsularly.

By 15 weeks p.i., the lens capsule of many cataractous lenses has undergone in-
creased proliferation to reach roughly 10 times normal thickness (Fig. 8). After the
fourth week p.i., both the retina and cho-
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Fig. 7. Four weeks p.i.: The lens becomes ovoid in shape, with folding of the redundant capsule, due to resorption of the lens substance. (Periodic acid-Schiff; x40.)

Fig. 8. Fifteen weeks p.i.: Lens capsule undergoes generalized lamellar proliferation, with subcapsular nodule formation, and achieves roughly 10 times its normal thickness. (Periodic acid-Schiff; x330.)

Bio: In certain areas to as little as one cell layer in thickness, with minimal to absent inflammatory infiltrate. Throughout the course of the disease, in most cases, pathologic changes occur in both eyes of the same animal, with the same rate and intensity.

Eyes of the control animals do not exhibit any of the pathologic changes noted in the experimental group. Among mature animals of identical age, the diseased eyes are considerably smaller in size than the control eyes.

Discussion

The SMCA eye pathology exhibits a distinctive pattern composed of generalized inflammation, or endophthalmitis with early retinitis, leading to posterior uveitis. This is followed by degenerative changes and, in the case of the lens, also reparative or proliferative features. However, in approximately 20 per cent of all cases after 3 weeks p.i., lens necrosis is extensive enough to lead to capsule breakage, resulting in a foreign-body granulomatous reaction. This granulomatous picture corresponds to a pattern consisting of severe, peribulbar inflammation, progressing to phthisis bulbi, which Clark described in 31.3 per cent of Wistar rats, 60 to 90 days p.i.

The results of the present study are consistent with the general course of the histopathology described by previous investigators but, in addition, include new detailed findings. However, the progressive development of the histopathologic features reported in the current study does not correlate readily with the slit lamp changes in SMCA-infected mice described by Olmsted and associates.

It has been well established that lens epithelium is activated to undergo rapid proliferation as a result of numerous types of external stimuli. The lens epithelial transformation into a multilayer resembles changes described in other models of cataract formation, including human senile and anterior polar cataracts, several experimentally induced cataracts of noninfec-
tious etiology, and a hereditary cataract of mice. These models also may have associated basement-membrane changes, similar to the SMCA-induced cataract. However, increased accumulation of basement-membrane material, without proliferation of the lens epithelium, has been noted in the coronary cerulean cataract of Mongolism and the cataract of Lowe's syndrome. The basement-membrane changes in the SMCA-induced cataract may be secondary to the increase in the number and/or productive capacity of the epithelial cells, from which this material is derived. Persistence of nuclei among cells at the bow region and appearance of hydropic lens cells are also features shared by cataracts of other etiologies.

The extension of lens cells to the posterior aspect of the lens is similar to that noted in radiation-induced cataract of rabbits. Yet this feature in the SMCA-related cataract is of much greater severity. Continued production of anterior lens epithelium, perhaps stimulated to proliferate even faster than normal due to injury, apparently forces in a posterior direction the nonelongating cells of the equator. Studies in the chick embryo indicate that failure of elongation of lens cells at the bow is related to damage of the neural retina.

In the early phase of the disease, lack of lens change in the presence of striking retinitis suggests that the main pathologic process is inflammation in the posterior eye tissues. This is reinforced by work of Clark and Karzon on recovery of agent from individual eye compartments. Their studies indicate that agent is present earlier and reaches a higher titer in retina than in any other eye tissue.

Pathology in the lens may therefore be the result of the primary inflammatory process occurring elsewhere in the eye. Intraocular inflammation alone has been widely recognized to cause secondary cataractous transformation of the lens. The name, "suckling mouse cataract agent," derived from early laboratory observations, might mislead one to wrongly assume that the agent has a specific affinity for the lens, and directly causes cataracts.

It is unlikely that SMCA which is introduced into animals after term could actually penetrate the relatively impervious lens capsule, and thereby grow inside it to produce the observed pathology. Viral agents such as rubella, mumps, and herpes, which actually grow in the lens, are known to enter the lens only very early in fetal life before the lens pore has closed. SMCA recovered by Clark and Karzon from pooled-lens tissue may have merely represented organism attached to the external surface of the lens capsule. To confirm whether or not SMCA actually grows in the lens will require future studies, for example, direct visualization by means of electron microscopy of organism in lens tissue of infected animals.

The character of the pathologic changes in all cases was uniform in nature. However, variations in severity and time course of the features did occur, probably secondary to idiosyncratic response of each experimental host, or unintentional fluctuations in the amount of inoculum administered. Although ultimately cataract production results in nearly 100 per cent of experimentally treated rats, the variability in both severity and time course places some restrictions on the use of the SMCA-induced eye disease as a model for study of cataractogenesis.

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REFERENCES