Macrophage Mediated Damage to Rat Lenses in Culture: 
A Possible Model for Uveitis-associated Cataract

J. Samuel Zigler, Jr., Igal Gery, David Kessler, and Jin H. Kinoshira

Rat lenses incubated in the presence of “activated” murine peritoneal macrophages are markedly impaired in their ability to accumulate certain radio-labeled compounds from the culture medium whereas incubation with resident macrophages has no such effect. The damage to the lens can be prevented in part by addition of certain antioxidants to the culture medium. The results suggest that mediators released by activated macrophages may be involved in initiation of the cataracts associated with chronic ocular inflammation. Invest Ophthalmol Vis Sci 24:651-654, 1983

Cataract is a frequent secondary complication of chronic uveitis. Such cataracts have classically been called “complicated cataracts” and typically originate in the posterior subcapsular area. The mechanism of initiation of these cataracts is uncertain. It has been suggested that elevated lyssolecithin present in the aqueous humor as a result of breakdown of the blood-aqueous barrier may act as a cataractogenic factor; however, our experimental data do not support this notion (to be reported elsewhere). Another possibility is that compounds released by the inflammatory cells concentrated in the uveitic eye may initiate cataract formation. In chronic uveitis most of the inflammatory cells are macrophages (Mφ), which are known to release numerous potential mediators, including a variety of hydrolytic enzymes, prostaglandins, complement components, monokines and reactive metabolites of oxygen. Among the reactive oxygen species produced are superoxide (O2·−), H2O2, OH·, and probably singlet oxygen (1O2), all of which have been implicated in the cytotoxic and cytotstatic effects of macrophages on a variety of cell types including tumor cells and bacteria. Since it has also been suggested by a number of laboratories that active forms of oxygen may play a role in the formation of certain types of human senile cataracts, we have investigated the possibility that activated oxygen species released by macrophages might be involved in the formation of the cataracts associated with chronic ocular inflammation. To this end the damaging effects of macrophages on lenses in organ culture were assessed by following the accumulation of substances normally concentrated by the lens.

Materials and Methods. Male BDF1 mice, 7–12 weeks old, were used without treatment (for “resident” Mφ), or after intraperitoneal injection with killed Corynebacterium parvum bacilli (Wellcome), 1.4 mg per mouse (for “activated” Mφ). After 9–14 days the mice were killed, and the peritoneal cells were collected by lavage, spun down, and resuspended in TC-199 medium with 10% fetal calf serum to the concentration of 5 × 106/ml resident cells or 2.5 × 106/ml activated cells. The peritoneal cells were cultured in multiwell plates (Linbro), 1 ml/well for ½–2 hrs at 37 C with 5% CO2, and the nonadherent cells were removed by thorough washing. Monolayers of lens epithelial cells were used as controls. Phorbol myristate acetate (PMA) activation was by incubation of monolayers with 1 μg/ml PMA for ½ hrs, followed by thorough washing.

Lenses excised from Sprague-Dawley male rats (50–75 g) were immediately put into individual tissue culture wells containing 1.5 ml of modified TC-199 at 295–300 milliosmoles, with or without cells prepared as outlined above. Incubation was at 37 C in 5% CO2, generally for 20 hrs. Four hours before termination of incubation, lenses were pulsed with tracer amounts of labeled compounds (3H-choline chloride, 80 Ci/mmole; 86RbCl, 10 Ci/gm; 14C-a-aminoisobutyric acid, 50 mCi/mmole; all from New England Nuclear). When 3H-choline was used, cold choline chloride was also added to a concentration of 50 μM.

After incubation lenses were removed, rinsed in saline, weighed, and homogenized in 1.0 ml of 10% TCA. Following centrifugation, aliquots (0.1 ml) of the TCA extracts and the culture medium for each lens were analyzed by liquid scintillation counting. Lens to medium ratios for radio-labeled compounds were calculated assuming water content of the lenses to be 62% of wet weight. For control lenses L/M for 86Rb ranged from 6–8 and for 3H-choline from 5–10 in the various experiments. In the control lenses the ability to accumulate the labeled compounds was stable over the 20-hr incubation period.

Results. Figure 1 demonstrates that cultured lenses exposed to PMA-activated Mφ are impaired in their ability to accumulate certain radio-labeled compounds. The impairment was observed consistently, although its magnitude was somewhat variable probably due to variation in the nature or extent of Mφ activation. Accumulation of choline was generally more sensitive to the effects of activated macrophages than accumulation of 86Rb. Accumulation of the amino acid 14C-a-aminoisobutyric acid was also damaged in this system to an extent comparable to that for 86Rb (data not shown). Macrophages elicited in vivo with C. parvum but not subsequently exposed to PMA had no significant effect on cultured lenses.
Radiolabelled Compound | Cells Added | PMA** | 25 | 50 | 75 | 100%
--- | --- | --- | --- | --- | --- | ---
$^3$H-Choline | None | - | | | | |
Activated Mφ | - | | | | | |
Activated Mφ | + | | | | | |
Resident Mφ | + | | | | | |
 Lens Epithelial Cells | + | | | | | |
Rb | None | - | | | | |
Activated Mφ | + | | | | | |
 Lens Epithelial Cells | + | | | | | |

*L/M (Percentage of Control)

Likewise, neither Mφ from untreated mice ("resident" Mφ) nor lens epithelial cells treated with PMA produced detectable lens damage. Lens epithelial cells were used as a nonmacrophage control cell type.

A variety of agents that might potentially block the action of various mediators released by PMA-activated Mφ were added to the cultures in an attempt to identify the particular Mφ product(s) responsible for the observed lens damage (Fig. 2). Scavengers of H$_2$O$_2$ (catalase) and O$_2^-$ (superoxide dismutase) did not protect the lenses; likewise scavengers of OH$^-$ (mannitol) and O$_2^*$ (histidine) had no effect. Protection by the nonspecific antioxidants dithiothreitol and glutathione was observed consistently, although the extent of protection varied with different Mφ preparations. Indomethacin, a cyclo-oxygenase inhibitor, had no effect and neither did the steroid anti-inflammatory drug prednisolone. In addition, protease inhibitors were used but gave no protection.

To confirm that active oxygen species can damage lenses in culture, we exposed lenses to the well-studied xanthine-xanthine oxidase radical generating system, which is known to produce both O$_2^*$ and H$_2$O$_2$. Figure 3 demonstrates the very strong effect of this system on the lens' ability to accumulate choline. It appears that the active species is H$_2$O$_2$ since catalase is capable of completely protecting the lens under the conditions employed while scavengers of other forms of activated oxygen are ineffectual. The nonspecific antioxidant dithiothreitol also gave considerable protection; the failure of glutathione to protect probably results from the lability of its reduced form.

**Discussion.** Lens biochemists have for a number of years speculated that certain cataractous lens changes might be the result of oxidation by active species of oxygen. Indeed it has been shown previously that lenses in vitro are damaged by systems generating various forms of activated oxygen, including singlet oxygen, H$_2$O$_2$ and superoxide.$^{4-6,10}$ The data from the xanthine-xanthine oxidase system presented above also demonstrate that profound damage...
Fig. 3. Effects of an oxygen radical generating system on accumulation of $^3$H-choline by cultured rat lenses. All lenses were incubated for a total of 20 hrs in medium made 1 mM in xanthine. Each bar represents data on four to eight lenses used in one representative experiment. Multiple experiments were performed for each set of conditions. *L/M is the lens to medium ratio for $^3$H-choline.

<table>
<thead>
<tr>
<th>Xanthine Oxidase (0.018 U/ml)</th>
<th>Antioxidant</th>
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<td>+</td>
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<td>Superoxide Dismutase (300 U/ml)</td>
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<td>+</td>
<td>Catalase (10,000 U/ml)</td>
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<td>+</td>
<td>Glutathione (1 mM)</td>
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<td>+</td>
<td>Dithiothreitol (1 mM)</td>
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<td>+</td>
<td>Vitamin E (100 µg/ml)</td>
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<td>+</td>
<td>Mannitol (15 mM)</td>
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<tr>
<td>+</td>
<td>Histidine (15 mM)</td>
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*L/M (Percentage of Control)

25 50 75 100%


to cultured lenses can result from oxidants produced outside the lens.

It is reasonable, therefore, to expect that the MΦ present in a chronically uveitic eye would produce an oxidative stress on the lens, since such MΦ are known to release activated forms of oxygen and to have marked cytostatic and cytotoxic activity. The protection afforded by nonspecific antioxidants such as glutathione and dithiothreitol indicates that oxidation is involved in the damage to cultured lenses following exposure to MΦ. The requirement that the MΦ be exposed to PMA in order to damage cultured lenses is also consistent with this view. It has been shown that in vitro, activation with PMA is required to stimulate the MΦ oxidative burst, ie, the intense production of activated species of oxygen. While both the activated MΦ and the xanthine-xanthine oxidase system are known to produce superoxide and $\ce{H_2O_2}$ and while lenses exposed to each system underwent marked damage, our data clearly show that only in the xanthine oxidase system did a specific scavenger (catalase) of an activated oxygen species protect the lens. The inability of the specific scavengers of $\ce{H_2O_2}$, $\ce{O_2^-}$, $\ce{OH^-}$, or singlet oxygen to counteract the damaging effect of macrophages may indicate that the oxidative damage is not due to a direct effect of these activated species. However, it is also conceivable that the macrophages affect the lens only by contact or at close proximity, thus reducing the efficacy of the scavengers.

In conclusion, it is clear that activated MΦ are capable of damaging lenses in vitro. The criterion used to assess lens damage was impairment of the ability of the lens to accumulate substances normally concentrated by the lens. Since our aims were to determine if MΦ could damage the lens and, if so, what the toxic substance(s) was, we have not characterized the precise nature of the damage in terms of uptake or leak-out mechanisms. The observed damage is similar to that found in cortical cataracts in vivo in that the lenses are unable to maintain normal levels of cations and certain nutrients and ultimately undergo osmotic swelling and loss of transparency. The mechanism of action of the MΦ in this system is not clear and probably is complex since these cells are releasing such a variety of potential mediators. Our use of a cyclo-oxygenase inhibitor (indomethacin), an anti-inflammatory steroid (prednisolone), and protease inhibitors all failed to yield evidence that the types of mediators against which these agents were directed play a role in the lens damage. Still it must be stressed that activated MΦ release such a variety of potential mediators, undoubtedly including many not yet recognized, that it is quite conceivable that factors other than oxidants participate in the damage seen in our system. Our data do clearly indicate, however, that an oxidant is involved in macrophage-induced lens damage in vitro. The possibility that a similar process contributes to the cataractogenesis associated with chronic uveitis is intriguing, but remains to be proven.

**Key words:** rat lens, transport processes, macrophage, phorbol myristate acetate, uveitis, inflammation, oxidants, xanthine-xanthine oxidase
Various types of hereditary retinal degeneration have associated posterior subcapsular cataract (PSC). It has been claimed that in the Royal College of Surgeons (RCS) rat model of hereditary retinal dystrophy, the cataract is manifested unpredictably and does not display Mendelian inheritance. It was shown previously, however, that 100% of pink-eyed retinal dystrophic RCS rats had an onset of bilateral PSC at 7 to 8 weeks of postnatal age, and by 9 to 11 months, 23% of the animals had cataracts visible to the unaided eye. The congenic black-eyed RCS rats fed a natural ingredient NIH rodent diet had 100% of black-eyed rats (3%) by 2.5 to 11 months of age, 3 but the black-eyed rats were shown previously to have a much lower incidence of mature cataracts (3%) by 2.5 to 11 months of age, 3 but the age of onset was not studied. Black-eye pigmentation initiated in the PSC, its influence was not decreased by dark pigmentation of the eye. RCS rats may be a model for an early onset type of human autosomal recessive retinal degeneration having a constant association of PSC. Invest Ophthalmol Vis Sci 24:654-657, 1983

Posterior subcapsular cataracts (PSC) are often seen in association with retinal degenerations, such as retinitis pigmentosa and gyrate atrophy of the retina and choroid. They also occur in the Royal College of Surgeons (RCS) rat model of autosomal recessive retinal dystrophy. The relation of PSC to retinal degeneration is not understood. Recently we demonstrated in pink-eyed, tan-hooded dystrophic RCS rats fed a natural ingredient NIH rodent diet that onset of PSC was between 7 and 8 weeks of age, at which time bilateral sugar grain-appearing opacities were distinguishable by slit-lamp examination. All rats examined at later ages had PSC, although only 23% of them developed mature cataracts by 9 to 12 months of age. This demonstrated that the PSC of the RCS rat occurred in a predictable fashion and at about the time when most of the rod photoreceptors had degenerated (about 2 months).

The congenic black-eyed retinal dystrophic RCS rat may be a better model for some type of hereditary retinal degeneration in humans, most of whom have pigmented eyes. The black-eyed rats were shown previously to have a much lower incidence of mature cataracts (3%) by 2.5 to 11 months of age, but the age of onset was not studied. Black-eye pigmentation

References


Slit-lamp Assessment of Onset of Cataracts in Black-eyed, Black-hooded Retinal Dystrophic Rats

Helen H. Hess, David A. Newsome, Joseph J. Knopko, and Gloria E. Westney