Postinflammatory cataracts in the mouse: induction by human mycoplasma-like organisms

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Abstract

Cataracts often occur in humans secondary to uveitis. Uveitis may be caused by various infectious agents, but rarely is the agent detected in the cataract. Mycoplasma-like organisms (MLO) were recently reported to cause human uveitis and retinitis. Cataracts were often present in those inflamed eyes. MLO are intracellular cell wall deficient pathogenic bacteria. They are pleomorphic tubulospherical and filamentous organisms with a characteristic ultrastructural appearance. No MLO culture system has been found despite 20 years of effort. The diagnosis of MLO disease rests on detection of the organisms in parasitised cells by a transmission electron microscope and response to antibiotics. In human intraocular inflammatory disease MLO are detectable in parasitised leucocytes and retinal pigment epithelial cells at the disease sites. Inoculation of MLO from a human source into mouse eyelids produced intraocular, chronic, progressive, inflammatory disease, with intraocular leucocytes parasitised by MLO in 15 of 100 mice versus 0 in 200 controls ($p < 0.05$). This report describes the cataracts with MLO-parasitised intralenticular leucocytes in the inflamed eyes of 14 of those 15 mice versus 0 in 200 control mice ($p < 0.05$). The results indicate that MLO penetrated the lens capsules to produce the cataracts, and they suggest that MLO could cause human cataracts. Alternative methods for detection of MLO and rifampin treatment of MLO intraocular disease are discussed.

Cataracts often occur in humans as a result of intraocular chronic inflammatory disease, in both idiopathic intraocular inflammatory disease and inflammation caused by specific infectious agents.^{1,2} The mechanism of postinflammatory cataract formation is complex and poorly understood, and only rarely is an intralenticular infectious agent found.^{1,2} Mycoplasmas are cell wall deficient bacteria assigned to class Mollicutes.³ Mollicutes ('mycoplasma') stain poorly with the usual biological stains and pass bacteria-retaining filters.⁴ Mycoplasmas may be overlooked or confused with viruses.⁴ They are pleomorphic tubulospherical and filamentous organisms with a characteristic ultrastructural appearance,⁴ and they exist as extracellular and intracellular forms.⁵ *Spiroplasma mirum*, an intracellular mollicute, produces inflammatory cataracts in young mice.⁶ Other intracellular mycoplasmas are a well studied cause of chronic disease in plants.⁷ Most plant intracellular mycoplasmas remain uncultivated despite over 20 years of effort.⁷ Lacking a culture system, these

forms are called 'mycoplasma-like organisms' or simply MLO.⁷ Diagnosis of MLO disease rests on direct detection of these organisms in parasitised cells by transmission electron microscopy.⁷

MLO were recently reported to cause human uveal, retinal, and orbital chronic inflammatory disease with autoimmune features.⁸⁻¹¹ Cataracts were often present in these inflamed eyes.⁸⁻¹¹ In human MLO intraocular disease these pathogens have been reported in parasitised retinal pigment epithelial cells¹² and vitreous and/or aqueous leucocytes.⁸⁻¹¹ All leucocyte cell lines, including lymphocytes, monocytes, and polymorphonuclear leucocytes, may undergo MLO parasitisation.⁸⁻¹⁶ Cells parasitised by MLO display distinctive fine structural alterations in cytoplasmic organelles and nuclei.^{5,7-17} The nuclear alterations are probably the result of MLO nucleases¹⁸ and expropriation of host cell nuclei acids by MLO.¹⁶ Parasitisation by MLO results in host cell dysfunction, proliferation, and/or destruction.^{7,8-17,19} MLO can be transmitted.⁷ Inoculation of human ocular disease MLO into the eyelids of young mice produced intraocular inflammatory disease in 15 of 100 mice versus 0 of 200 controls after a three-month latent period.²⁰ MLO parasitised leucocytes were present in the intraocular inflammation.¹⁴⁻¹⁶ Vasculitis was the initial lesion.²⁰ The disease was chronic and progressive.²⁰ Features of the progressive disease were extensive lymphocytic infiltrates, phthisis bulbi, exophthalmos, and cataracts.^{20,21} This report provides details of those cataracts.

Materials and methods

Four patients with intraocular chronic inflammatory disease served as sources of the MLO inocula. The clinical details are reported elsewhere.²² The inflamed vitreous from each patient was removed by vitrectomy.²² All specimens were available for laboratory studies by an approved protocol of the Investigational Review Board. No growth occurred from any of the vitreous specimens despite a large variety of culture techniques, and none of the specimens produced a cytopathogenic effect in various tissue culture cell lines.²² Each specimen showed MLO parasitised leucocytes on transmission electron microscopy.¹⁴⁻¹⁶ Each was stored at 4°C until animal inoculation.

Table 1 Incidence of cataracts by mouse group

MLO vitreous inoculated	Eye bank vitreous inoculated	Sterile saline inoculated	p
14/100	0/100	0/100	<0.05

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Figure 1 MLO induced mouse cataract. Section through the anterior chamber and lens displays extensive intraocular chronic inflammation and severe cataractous degeneration. The identity of the intralenticular leucocytes is apparent on higher magnification. (H-E, $\times 240$.)

The animal investigation was conducted in accordance with an approved protocol of the Animal Care Committee. The animals consisted of 300 CD/1 male mice, 12–16 weeks old and weighing 15–20 g at the time of inoculation. Approximately 0.1 ml of MLO specimen from each of the four patients was inoculated into the lateral aspect of each lower eyelid of 25 mice for a total of 100 test mice. Vitreous from each of 10 eye bank eyes was inoculated into 10 mice for a total of 100 control mice. A further control consisted of sterile saline inoculated into the eyelids of 100 mice. This gave a grand total of 200 controls. All mice were observed twice daily five days a week for 12 months for visible persistent eye disease and spontaneous death. The results of those observations have been reported.^{20 21} Three MLO inoculated mice with visible persistent ocular disease and three mice inoculated with eye bank vitreous were killed during observation months 4–7 by means of carbon dioxide in a closed container. Mice surviving at the end of 12 months were similarly killed.

The eyes and orbital tissue of all mice were exenterated, fixed in 10% formalin, and processed for routine haematoxylin-eosin stained tissue sections. Under a light microscope all eyes were examined for cataracts, and the incidence of cataracts by mouse was group calculated and compared. The relationship between cataracts and intraocular chronic inflammation was also determined. In addition portions of one lens from each of three MLO inoculated and three eye-bank inoculated mice killed during observation months 4–7 were finely minced, fixed in

4% glutaraldehyde, washed in 0.1 M cacodylate buffer, postfixed in osmium tetroxide, and embedded in Araldite. After polymerisation 1.0 μ m thick sections were stained with toluidine blue and studied for cataracts under a light microscope. Sections displaying cataracts were ultrathin sectioned, stained with uranyl acetate-lead citrate, and studied for MLO parasitised intralenticular leucocytes.

Results

HISTOLOGICAL STUDIES

The incidence of cataracts by mouse group is shown in Table 1. The cataracts were bilateral in 11 mice and unilateral in three. All the cataracts were profound. They were peripheral in 19 lenses and diffuse in six. The cataracts were present only in eyes with intraocular inflammation and were associated with synechiae in 12 mice. The capsules appeared intact, but all 25 cataractous lenses showed varying numbers of intralenticular leucocytes (Fig 1). The leucocytes consisted primarily of lymphocytes, but a few monocytes, plasma cells, and polymorphonuclear leucocytes were also present.

TRANSMISSION ELECTRON MICROSCOPIC STUDIES

The lenses of all three MLO vitreous inoculated mice displayed cataracts with intralenticular inflammation. MLO were readily detected in many of these leucocytes (Fig 2). The MLO parasitised leucocytes usually showed lysis of cytoplasmic organelles, irregular frayed nuclear contours, and chromatin lysis with clumping (Fig 2). None of the control lens sections displayed intralenticular leucocytes or MLO.

Discussion

Nearly every mouse eye with intraocular inflammation had cataracts with intralenticular leucocytes, whereas none of the control eyes had cataracts or intralenticular leucocytes. MLO parasitisation of the intralenticular leucocytes was readily apparent by electron microscopy. The results indicate that MLO was the principal cause of the cataracts. Mollicutes contain various potent toxins.^{18 19} The pathogenicity of MLOs in other tissues is grossly apparent and readily detectable by light and electron microscopy.^{5 7-17 20 21 23} Tissue and cell lysis are characteristic features of MLO disease.^{5 7-17 20 21 23} The smallest MLO particles measure 0.005–0.010 μ m.^{5 7-17} These particles are believed to be the ones that initiate cellular infection.¹⁴⁻¹⁶ They penetrated the capsule with no apparent histological damage. Thereafter the larger MLO forms developed and persisted within the cataract. Ingress of leucocytes probably occurred through minute foci of capsular damage. The intralenticular inflammation was a response to both MLO and lens protein. Intralenticular leucocytes undoubtedly contributed further to the cataractous changes.

Postinflammatory cataracts often occur in patients with MLO intraocular disease.^{8 12} Such cataracts are extracted and are available for

Figure 2 MLO parasitised intralenticular leucocyte. Innumerable MLO fill the cytoplasm of an intralenticular monocyte from a cataractous lens (arrows). (Uranyl acetate-lead citrate stain, $\times 23\,000$.)

laboratory study. From this investigation one would predict that MLO will be the pathogenic agent within these cataracts. It is noteworthy that intralenticular leucocytes are occasionally found in idiopathic postinflammatory cataracts.² The resemblance of these cataracts to MLO induced mouse cataracts is interesting.² MLO could explain the presence of the intralenticular leucocytes, and these could be readily studied for MLO.

Most human postinflammatory cataracts show no intralenticular inflammation.^{1,2} MLO could also cause such cataracts. With minimal MLO intraocular inflammation and capsular damage leucocytes may not invade the lens. In this situation MLO alone could produce a cataract by parasitising and destroying lens tissues.^{21,23} Detection of MLO by electron microscopy is difficult in the absence of leucocytes,^{14,16} and other techniques could be valuable.^{7,24} Prokaryotic cells often contain unique lipoidal substances not found in animal cells.²⁴ Sensitive assays for these substances are readily available.²⁴ The presence of such intralenticular substances

would indicate a bacterial, possibly MLO, aetiology for the cataract. Detection of MLO in human cataracts would have diagnostic and therapeutic implications.

Rifampicin (rifampin) is beneficial in human and experimental animal disease caused by MLO.^{8-12,25} It is a well tolerated antibiotic safe for long-term administration.²⁶ Its efficacy in MLO disease may be due partly to its active incorporation into leucocytes²⁷ and its possible inhibition of host cell nucleic acid expropriation by MLO.¹⁶ Early rifampicin therapy would be expected to be beneficial in treating human MLO intraocular inflammation and the associated cataract.²⁸

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