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Lens epithelium supports axonal regeneration of retinal ganglion cells in a coculture model in vitro

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Abstract

The purpose of this study was to determine whether the lens epithelium influences the survival or axonal growth of regenerating retinal ganglion cells. The optic nerves of adult albino rats were injured in order to induce axonal regeneration, and axon growth was then studied in retinal explants in the presence of cocultivated lens capsules carrying living epithelial cells. In the first series of experiments, cocultivation of retinal explants with lens epithelium in immediate proximity resulted in penetration of fibers into the lens epithelium, indicating that it supported axonal growth. In the second series of experiments, coexplants were placed 0.5–1.0 mm from each other. The numbers of outgrowing retinal axons were determined both with respect to the retinal eccentricity and the topological relationship with the lenticular coexplant. The Wilcoxon matched-pairs signed-rank test was used to determine if the numbers of axons differed significantly between four regions of the explants. Significantly more axons grew out from the retinal edge facing the lenticular explant than from its opposite side, indicating that the lens epithelium supports axon growth. The numbers of surviving retinal ganglion cells in culture were determined after retrograde prelabelling with a neuroanatomical tracer. The number of fluorescent ganglion cells within the retinal explants did not significantly differ between the groups (Mann–Whitney test). These findings indicate that the lens epithelium influences both the amount of axonal regeneration and the direction of growth without affecting the survival rate of retinal ganglion cells in vitro.

Keywords: retinal ganglion cells; lens epithelium; apoptosis; regeneration; growth factors; LEDGF; crystallins

1. Introduction

The primary visual pathway of rodents is a useful model for investigating injury-induced responses of neuronal cells in vivo and in vitro (Ramón y Cajal, 1928; Berry, 1982; Berry et al., 1996, 1999). The optic nerve (ON) is a typical unidirectional and long white-matter tract which, after cut or crush injury, does not support the regeneration of axons distal to the injury. The inability of retinal ganglion cells (RGCs) to regenerate their cut axons has been attributed to inhibitory influences of oligodendrocytes, scar-forming astrocytes, extracellular matrix proteoglycans, and lack of neurotrophins (Berry, 1982; Schwab et al., 1993; Berry et al., 1996, 1999). An axotomising injury to the intraorbital segment of the adult mammalian ON results in frustrated attempts of axonal sprouting and subsequent degenerative cascades, which result in secondary or delayed death of the RGCs (Ramón y Cajal, 1928). Recently, it has been shown that axotomised RGCs respond to a concomitant lens injury with prolonged survival and regeneration in vivo (Fischer et al., 2000; Leon et al., 2000; Yin et al., 2003) and in culture (Fischer et al., 2000; Lorber et al., 2002). Although, the effects of lenticular injury have been attributed to secondary inflammation and the release of neuroprotective macrophage-derived factors (Yin et al., 2003), the lenticular factors causing this cascade have not yet been identified.

The lens epithelium is ontogenetically and functionally a peculiar intraocular tissue with the unique feature of performing incomplete cellular apoptosis throughout life. The ectodermally derived epithelial cells permanently divide to produce the nuclei- and organelle-free lens-fiber cells that account for the optical transparency (Kuwabara and Imaizumi, 1974; Bassnett and Mataic, 1997). Numerous growth factors such as FGF1, FGF2, and IGF-1 have been

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Bcl-2	B-cell lymphoma-2
BDNF	brain-derived neurotrophic factor
CD	side of the central part of a retinal explant which opposes the cocultivated lens epithelium
CNTF	ciliary neurotrophic factor
CP	side of the central part of a retinal explant which faces the cocultivated lens epithelium
DAPI	4′,6-diamidino-2-phenylindole
4-Di-10-ASP	4-(4-(didecylamino)styryl)-*N*-methylpyridinium iodide
FGF	fibroblast growth factor
GDNF	glial-derived neurotrophic factor
GFAP	glial fibrillary acidic protein
IGF	insulin-like growth factors
LEC	lens epithelium cell
LEDGF	lens epithelial-derived growth factor
NGF	nerve growth factor
ON	optic nerve
PD	side of the peripheral part of a retinal explant opposite the cocultivated lens epithelium
PP	side of the peripheral part of a retinal explant facing the cocultivated lens epithelium
RGC	retinal ganglion cell
SMA	smooth muscle actin
TGF	transforming growth factor

identified within the lens (Caldes et al., 1991; Schulz et al., 1993), and the antiapoptotic molecule Bcl-2 has been identified within the lens epithelium (Bonfanti et al., 1996; Wride et al., 1999), whereas typical neurotrophic factors such as NGF, CNTF, BDNF, and GDNF are not detectable at the protein level (Fischer et al., 2000).

Quantifying the remarkable neuroprotective and neuritogenic activity exerted by the injured lens on RGCs (Fischer et al., 2000; Leon et al., 2000; Lorber et al., 2002; Yin et al., 2003) may help identify the proteins accounting for the prevention of cell death. Identification of such factors may also be crucial to an understanding of pathways for blocking apoptosis in nonlenticular systems. One possible mechanism is that lenticular factors activate macrophages which subsequently support axonal growth (Leon et al., 2000; Yin et al., 2003; Fischer et al., 2004).

The outgrowth of axons in cultures of dissociated RGCs (Lorber et al., 2002) or retinal pieces is significantly improved by either prior or simultaneous cataractogenic injury of the lens in vivo. The associated experiments, however, did not exclude indirect effects of the surgical procedure of lens injury or subsequent release of lens fibers that may operate through inflammation and activation of macrophages. Such effects can only controlled for in coculture models of retinal tissue and isolated lens epithelium. In the present study, we developed such a model employing cocultures of adult rat retinal stripes and primary lenticular epithelium. In this model, we examined whether (1) the growth of RGC axons was supported on the lenticular epithelium (adjacent coexplants), (2) the lens epithelium attracts outgrowing retinal axons, (3) the lens epithelium enhances the number of regenerating retinal axons, and (4) the lens epithelium supports survival of RGCs in cocultured retinal explants.

2. Materials and methods

2.1. Surgical procedures

All experiments were performed using adult Sprague–Dawley rats of both sexes. The care and maintenance of the animals conformed to the ARVO Statement of the Use of Animals in Ophthalmic and Vision Research. For anaesthesia, the animals received an intraperitoneal injection of a mixture of 10 mg of ketamine sulfate (Sanofi-Ceva, Düsseldorf, Germany) and 1–2 mg of xylazine (Sanofi-Ceva) per 100 g body weight. To perform an open crush on RGC axons and thereby prime the growth of RGC axons in culture (Bonfanti et al., 1996), the ON was surgically exposed intraorbitally and, after longitudinal incision of the meninges, it was mechanically crushed for 10 sec at 1 mm from the globe using jewelers forceps under a surgical microscope.

This procedure was slightly modified in another experiment in order to visualise RGCs within the explants and determine their numbers in coexplants. For retrograde labelling, the fluorescent dye 4-(4-(didecylamino)styryl)-*N*-methylpyridinium iodide (4-Di-10-ASP, Molecular Probes, Eugene, OR) was applied to the ON, and the corresponding retinas were excised 3 days later for culture preparation.

As determined in previous studies, the RGCs have to be conditioned about 5 days prior to explantation in order to obtain prompt axonal growth in culture (Bahr et al., 1988; Bonfanti et al., 1996). This surgery avoids damage to blood supply and consequential ischaemic damage to the retina, because the central retinal artery and vein enter the retina within the nasal inferior meninges that are untouched by the procedure. At the end of surgery, retinal blood circulation was verified by fundoscopy. Five days later, the animals were killed by CO_2 inhalation to allow removal of the eyes and the production of explants.

2.2. Explantation and coculturing procedures

The anterior segment was removed from the globe by an circular incision centrally from the ciliary body (Fig. 1A). The lens within its capsule was separated from the ciliary body. A paraequatorial capsulotomy was performed and either the anterior capsule with the attached epithelium or the epithelium-free posterior capsule (in the control group) was