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Apoptosis in mammalian eye development: lens morphogenesis, vascular regression and immune privilege

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Abstract

Formation of the mammalian eye requires a complex series of tissue interactions that result in an organ of exquisite sensory capability. The early steps in eye development involve extensive cell death associated with morphogenesis. Later, suppression of programmed cell death is essential for tissue differentiation and in the adult, the immune privileged status of the eye is maintained in part through factors that induce inflammatory cell apoptosis. Experimental evidence suggests that suppression of apoptosis in cells of the lens lineage by fibroblast growth factors is one component of their action during lens morphogenesis. Fibroblast growth factors are also required for normal lens fiber-cell differentiation. This includes a degenerative step for organelles that is presumably an adaptation for the clearance of light scattering elements from the optic axis. The process of organelle degeneration may be related to apoptosis in a few of its features. Actively-induced apoptosis becomes important for eye development as the temporary ocular vasculatures regress. This too, is presumably an adaptation for the disposal of cells that would disturb the passage of light to the retina. Ocular macrophages appear to be essential for the induction of apoptosis in the endothelial cells comprising the ocular vasculatures. In the adult, inflammatory cells entering the eye are exposed to the pro-apoptotic agents transforming growth factor-β2 and Fas ligand. The expression of these molecules in the eye, and their action in killing inflammatory cells, has evolved as a means of preventing inflammation and subsequent loss of vision. Thus, the eye offers a unique and versatile system for studying the role of programmed cell death in lens development, vascular regression and immune privilege.

Keywords: programmed cell death, apoptosis, development, eye

Abbreviations: FGF, fibroblast growth factor; Rb, retinoblastoma; VEC, vascular endothelial cells; PM, pupillary membrane; TNFα, tumor necrosis factor α; ACAID, anterior chamber-associated immune deviation; MHC, major histocompatibility complex; TGFβ, transforming growth factor β; FasL, Fas ligand; HSV, herpes simplex virus

Introduction

Despite the value that our species places on the aesthetic appeal and sensory function of the eye, it is the non-essential nature of this organ that recommends it most highly as an experimental system. This characteristic of the eye has been exploited most fully in genetically manipulable organisms such as Drosophila where flies with mutated eyes are often viable and readily identified. The accompanying review from Nancy Bonini examines how cell death influences development of the Drosophila eye. In this instance, as well as in vertebrate systems, accessibility is also a critically important aspect of the ease with which the eye lends itself to experimental analysis.

The vertebrate eye is extremely well defined anatomically. The mature cell types that comprise this structure are both spatially and morphologically distinct and this characteristic also eases experimental analysis. The highly specialized mature cell types such as the lens and retina are relatively simple structures in which it is appealing to answer questions about the process of differentiation and development. The purpose of this review is to examine what normally occurring and experimentally induced programmed cell death can teach us about mammalian eye development and emphasizes the lens, ocular vasculatures and ocular immune privilege. The accompanying review from David Papermaster broadly examines the consequences of cell death in the eye for development and disease with emphasis on events occurring in the retina.

Programmed cell death during early eye development

The vertebrate eye begins its existence with a series of inductive events between neural and ectodermal tissues (Spemann, 1901; Grainger, 1992). Subsequently, the optic sulcus evaginates from the wall of the anterior neural tube and culminates in formation of the optic cup and the invagination of the region of the ectoderm that will become the lens (Figure 1). The retina begins its differentiation from the outer layer of the optic cup and the pigmented epithelium from the inner layer. Cells of the lens vesicle are, in turn, influenced to begin differentiation by stimuli from the surrounding environment (Coulombre and Coulombre, 1963) that probably have their origin in the neural retina (Yamamoto, 1976). Recent evidence from *in vivo* studies suggests that one of the stimuli is a fibroblast growth factor (Chow *et al*, 1995; Robinson *et al*, 1995b) and its action results in the elongation of crystallin-expressing lens fiber cells from the posterior hemisphere of the lens.

Early events in formation of the eye are associated with programmed cell death (Glucksman, 1951). As the optic sulcus extends medially to form the optic vesicle, neural crest-derived mesenchyme is situated between the distal

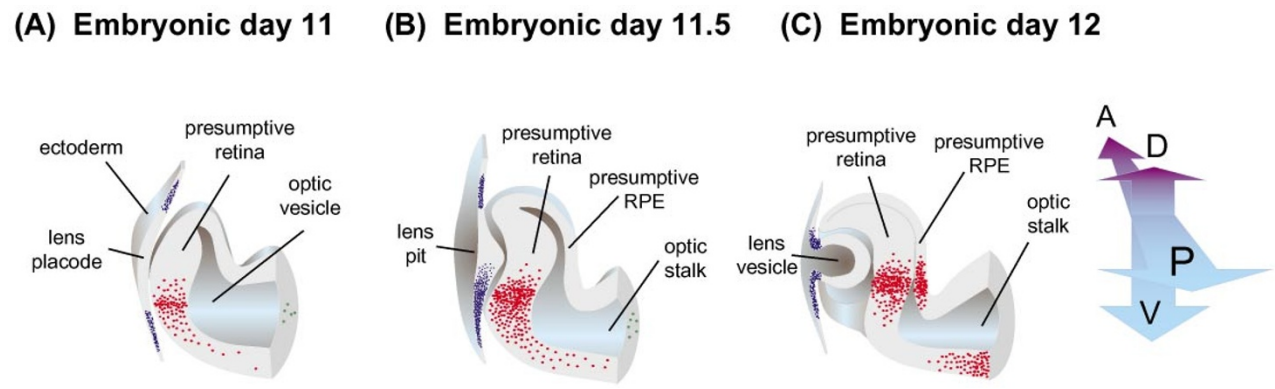

Figure 1 The pattern of cell death in the early developing eye. Pyknotic nuclei indicative of programmed cell death are observed in the components of the early rat eye between embryonic days 11 and 12 (**A**–**C**). Cell deaths occur in the ventral aspect of the presumptive retina and ventral and anterior walls of the optic vesicle (**A**–**C**, red dots and green dots). In the presumptive lens ectoderm, pyknotic nuclei (blue dots) are positioned in an annular region that circumscribes the area that will become the lens (**A** and **B**). Later, pyknotic cells are observed at the separation border between the lens vesicle and the surface ectoderm (**C**). The axes are indicated by the blue arrows at right (A, anterior. P, posterior. D, dorsal, V, ventral).

wall of the optic vesicle and the ectoderm. Ultimately, mesenchymal cells are excluded, and the optic vesicle makes direct contact with the ectoderm. Though disputed (Harch *et al*, 1978), it has been suggested that mesenchymal cells in this region are normally excluded through programmed cell death (Silver and Hughes, 1974). One might argue that programmed death of intervening mesenchymal cells may have evolved as a means of allowing the observed close contact between the optic vesicle and ectoderm. However, since close contact between the optic vesicle and ectoderm appears not to be essential for appropriate inductive interactions (Mckeehan, 1951; Muthukkaruppan, 1965), the suggestion that continued proliferation of these interstitial mesenchymal cells (and inhibition of contact) is the cause of anophthalmia in the ZRDCT mouse strain (Silver and Hughes, 1974) is difficult to sustain.

Pyknotic nuclei characteristic of apoptotic cells can be observed in the ventral wall of the optic stalk and in the presumptive lens ectoderm beginning at E11 in the rat (Figure 1) (Silver and Hughes, 1973). The dying cells observed in the optic cup do not die simultaneously but rather follow in temporal series from the disto-dorsal to the proximo-ventral pole of the eye rudiment. While there is no obvious rationale for cell deaths occurring in the optic stalk, in the ectoderm it is clearly associated with lens pit invagination. The area where apoptosis occurs in the ectoderm is annular in shape and circumscribes the region from which the lens will form. Later, at E12, apoptotic ectodermal cells are restricted to the boundary at which the lens vesicle is destined to separate from the surface ectoderm (Figure 1C) and one can suggest that here, apoptosis has evolved as a means of dissociating the two tissues (Silver and Hughes, 1973).

The lens

Lens rotation experiments performed in the chick some 30 years ago demonstrate that polarization of the lens (anterior epithelium and posterior fiber cell mass; Figure 2) is dependent upon the local environment. A lens that is rotated through 180° to place the lens epithelium adjacent to the vitreous will completely repolarize over a period of days (Coulombre and Coulombre, 1963). This observation has suggested that diffusible stimuli present in the aqueous and vitreous may be responsible for imposing polarization as well as the size and shape of the lens.

The presence of fibroblast growth factors (FGFs) 1 and 2 in retina conditioned medium (Richardson and McAvoy, 1986) and vitreous (Schulz *et al*, 1993) and the activity of these growth factors in stimulating fiber cell differentiation *in vitro* (Chamberlain and McAvoy, 1987, 1989) and *in vivo* (Chow *et al*, 1995; Robinson *et al*, 1995a, b) suggest a role in lens polarization.

Growth factor signal deprivation induces apoptosis of lens fiber cells

Recent analysis has aimed at determining whether FGF is required for the differentiation of lens fiber cells *in vivo*. Expression of a truncated form of an FGF receptor in cells that should respond to FGF is known to block signalling through the formation of non-functional receptor dimers (Ueno *et al*, 1992; Li *et al*, 1994). When such a dominant negative FGF receptor is expressed from a transgene in differentiating lens fiber cells, their elongation is impaired (Chow *et al*, 1995; Robinson *et al*, 1995a) and leads in some lines of transgenic mice to separation of the fiber cell mass from the posterior surface of the lens epithelium, a small lens, and associated microophthalmia. Biochemical demonstration that the FGF signal transduction pathway is inhibited in lens fiber cells (Chow *et al*, 1995) builds a very strong case that fiber cells are, at least in part, dependent upon FGF for their differentiation. These analyses largely confirm suggestions about the role of FGF in lens development inspired by *in vitro* studies (McAvoy *et al*, 1991).

An additional consequence of transgenic expression of a dominant negative FGF receptor in the lens was the death of lens fiber cells (Chow *et al*, 1995; Robinson *et al*, 1995a). Pyknotic nuclei and apoptotic bodies were

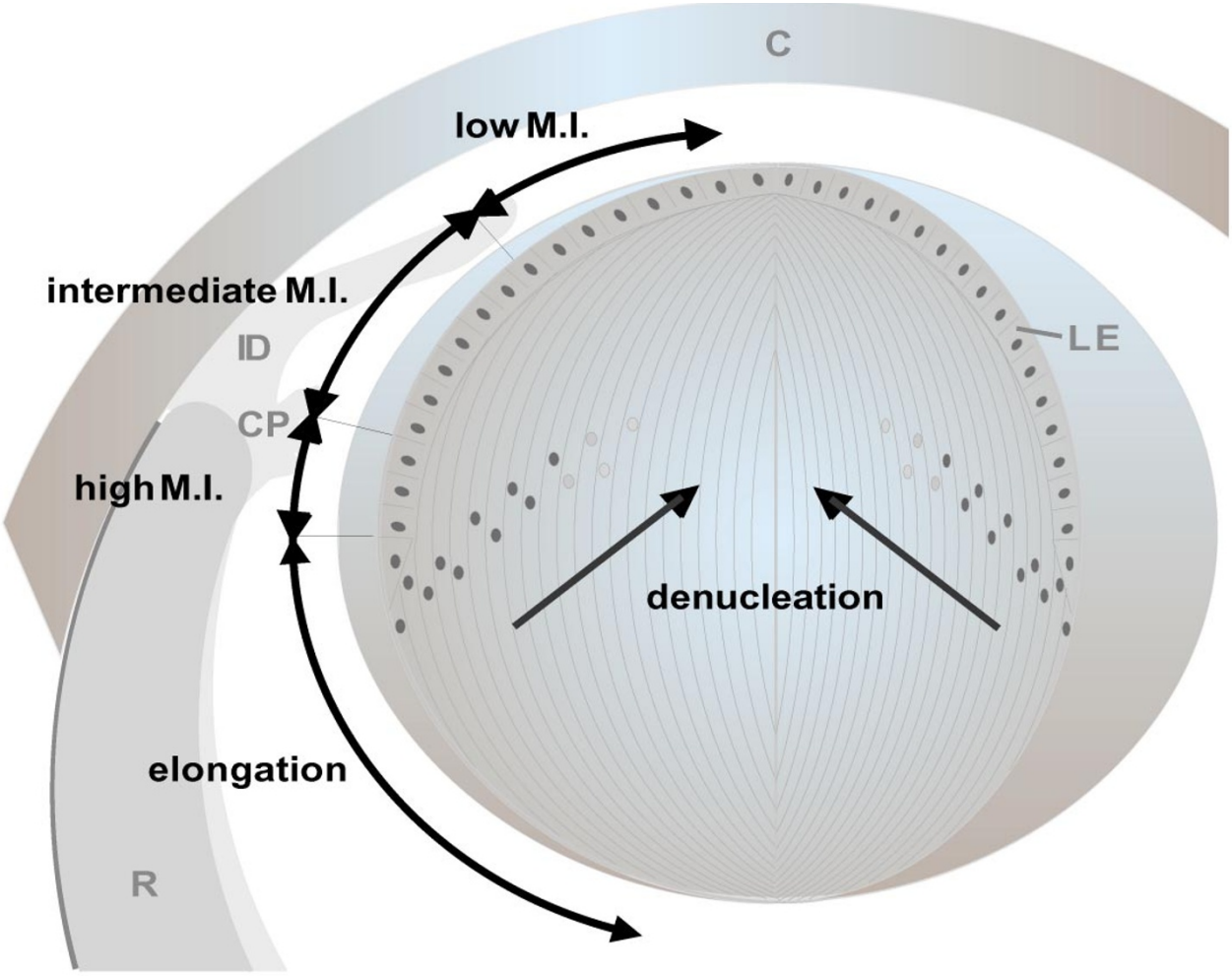

Figure 2 Regional compartmentalization of the lens. The position of a cell in the lens betrays its state of proliferation or differentiation. Proliferating cells are restricted to the lens epithelium (LE). Within the lens epithelium, regions of low, intermediate and high mitotic index (MI) can be identified. Differentiating lens fiber cells are confined to the interior volume and posterior hemisphere of the lens. As the fiber cells begin their differentiation, they elongate, express β- and γ-crystallin proteins and ultimately undergo the organelle degeneration that includes denucleation. The boundaries of the different compartments correspond roughly to anatomical features (R, retina. CP, ciliary process. ID, iris diaphragm. C, cornea).

observed in both severely (Robinson *et al*, 1995a) and mildly affected transgenic animals (Chow *et al*, 1995). Use of the TUNEL technique (Gavrieli *et al*, 1992) to label cells in lens sections indicated that fiber-cell death involved DNA fragmentation and confirmed that the cell death had characteristics of apoptosis. The most likely explanation for this observation is that lens fiber cells are dependent upon FGF for their survival as well as for their differentiation (Chow *et al*, 1995; Robinson *et al*, 1995a). This would certainly be consistent with an expanding literature documenting the survival stimulating activity of a variety of growth factors *in vitro* and *in vivo* (Angeletti *et al*, 1971; Raff, 1992; Campochiaro *et al*, 1996).

An alternative explanation for the apoptosis of lens fiber cells denied FGF signalling, is that the progressive development of an abnormal lens structure may result in apoptosis secondary to growth factor deprivation. This is probably a cause of fiber cell apoptosis in transgenic mice used for cell cycle analyses where a grossly abnormal lens is formed (Pan and Griep, 1994, 1995) and is a possibility in other cases. For example, in some transgenic lines expressing a dominant negative FGF receptor in the lens, fiber cells are denied adherence at their anterior tip to the posterior surface of the lens epithelium. Substrate deprivation-induced apoptosis has been noted in a number of cell types (Frisch and Francis, 1994), and occurs when an existing matrix-binding interaction is broken. This cause for fiber cell apoptosis is less likely than growth factor deprivation since in mildly affected lines of transgenic mice expressing a dominant negative FGF receptor in the lens, there appears to be no lack of fiber cell adherence to the epithelium at the anterior tip (Chow *et al*, 1995), and apoptosis still occurs. This would argue that adherence is not the critical survival element.

Cell cycle conflicts lead to lens cell apoptosis

The simplicity of the lens as a structure has lured investigators interested in cell cycle regulation. Cell cycle entry and exit as well as differentiation are regionally compartmentalized in the lens. This is a particular advantage for those interested in cell cycle events during development, since a simple morpholo-

gical examination of an altered lens structure can betray the nature of alterations in division and differentiation.

Polarization of lens structure is reflected in the spatially restricted responses of component cells. The differentiation of lens fiber cells is restricted to cells at the posterior surface while proliferation is limited to the lens epithelial cells covering the anterior hemisphere. Furthermore, the proliferative response of lens epithelial cells is quantitatively different in adjacent regions of the epithelium (McAvoy and Chamberlain, 1989). Epithelium opposite the ciliary process has the highest proliferative index and successively more anterior regions show successively reduced mitotic indices. Curiously, the boundaries between regions correspond roughly with anatomical features suggesting that the availability of mitotic stimuli may be regulated topographically (McAvoy *et al*, 1991).

The retinoblastoma (Rb) gene product plays an important role in regulating growth and differentiation (Buchkovich *et al*, 1989; Chen *et al*, 1989). Rb functions during G1 phase of the cell cycle by binding to and blocking the activity of the E2F family of transcription factors when it is in the active, hypophosphorylated state (Chellappan *et al*, 1991; Chittenden *et al*, 1991; Goodrich *et al*, 1991). Hyperphosphorylation of Rb by the cyclin-cdk complexes inactivates Rb and releases E2F to allow expression of such genes as N- and c-myc that promote entry to S phase of the cell cycle (Nevins, 1992). Two recent studies indicate that Rb has a function in cells of the lens lineage. In an extensive analysis, Pan and Griep (1994) used the human papilloma virus E7 gene product to block the function of Rb during the differentiation of lens cells. Transgenic mice expressing E7 from a lens-specific promoter showed an absence of fiber cell differentiation, and the accumulation of dividing and apoptosing cells. Essentially identical results were obtained with Rb null mice (Morgenbesser *et al*, 1994) although in this case, analysis could be taken only to embryonic day 14.5 due to the death of the embryos. These data imply that Rb is required for the appropriately regulated transition from cell division to differentiation in the lens.

In both systems in which Rb function has been prevented in the lens, it was also shown that at least in part, the occurrence of apoptosis is dependent upon the function of the tumor suppressor p53 (Morgenbesser *et al*, 1994; Pan and Griep, 1994). Apoptosis of lens cells is suppressed when either transgenic mice expressing E7 in the lens, or Rb null mice, are crossed with p53 null mice. Although the signal for p53 activation is not known, in this case, one possibility is that apoptosis is a consequence of a cell cycle conflict occurring during deregulated lens morphogenesis. These analyses indicate that cell cycle checkpoints are operational in cells of the lens lineage.

Is fiber cell denucleation related to apoptosis?

Normal development of the lens involves the degeneration of fiber cell organelles. Experimental manipulations that result in apoptosis of lens fiber cells serve to highlight the question of whether the normal degenerative process that occurs in the terminal differentiation of lens fiber cells is related to apoptosis.

A number of studies have shown that all organelles including nuclei and mitochondria of lens fibers degenerate (Bassnett and Beebe, 1992), but that the morphology of degeneration depends on the origin of the fiber cell. In the mouse, organelles of primary fiber cells undergo degeneration a few days after they have reached the lens epithelium at their anterior tip at about embryonic day 16 (Vrensen *et al*, 1991). The morphological features of primary fiber cell organelle degeneration include nuclear accumulation of small granules which condense to osmiophilic bodies in the nucleus and cytoplasm. Later, these bodies are found distributed at the anterior and posterior poles of the primary fiber cells (Vrensen *et al*, 1991). Subsequently, secondary fiber cells (which begin their elongation at the lens equator) undergo organelle degeneration as they are displaced towards the lens center. The morphology of nuclear degeneration appears distinct from that of primary fiber cells with a gradual fading of nuclear staining until nuclear and cytoplasmic substances are indistinguishable; condensed nucleolar fragments remain for some time (Kuwabara and Imaizumi, 1974).

The major morphological distinction between maturing lens fibers and apoptosing cells is, of course, that the fiber cell does not undergo the convolution and blebbing typical of a programmed death. The outline of the fiber cell is preserved as is the cytoplasmic contents during degeneration of the nucleus and other organelles. Despite these differences, there are a few features that might argue that fiber cell organelle degeneration is related to apoptosis. A study of nuclear degeneration in the mouse at the EM-level have noted condensation of nuclear material (Vrensen *et al*, 1991), a feature that appears more pronounced in fiber cells of the normal chick lens (Sanwal *et al*, 1986). However, the morphology of condensation in degenerating fiber cell nuclei is not entirely characteristic of apoptosis since the condensed chromatin that would normally be found marginated at the nuclear envelope is missing.

In the lens of the chick, nuclear degeneration is associated with DNA fragmentation. This was shown nearly 30 years ago through the use of terminal-deoxy transferase and DNA polymerase preparations (Modak *et al*, 1969; Modak and Bollum, 1972) on lens sections. Furthermore, DNA isolated from chick lenses shows a nucleosomal ladder pattern characteristic of many programmed cell deaths (Counis *et al*, 1989; Appleby and Modak, 1997). In apparent conflict (a species difference notwithstanding), recent analyses of the mouse lens using the TUNEL assay has not revealed DNA fragmentation in the fiber cell nuclei of wild type lens sections, but readily reveals fragmentation in sections from experimentally manipulated lenses where a characteristic apoptosis has occurred (Chow *et al*, 1995; Robinson *et al*, 1995a). Furthermore, even at the light microscopic level, nuclear degeneration during fiber cell apoptosis caused by the expression of a dominant negative FGF receptor appears to be of distinct morphology from the naturally occurring nuclear degeneration accompanying fiber cell maturation (Chow *et al*, 1995; Robinson *et al*, 1995a). Thus, while there are a number of clear distinctions between apoptosis and fiber cell degenerative steps, the possibility remains

that some aspects of the mechanics of apoptosis might have been appropriated as an adaptation to the clear light scattering organelles from the fiber cell cytoplasm.

Cell death during ocular capillary regression

In mammals, once the basic pattern of the eye has been established, a network of capillaries known as the hyaloid system grows into the presumptive vitreous and onto the surface of the lens. The hyaloid system consists of three components that can be distinguished anatomically, the tunica vasculosa lentis (found on the posterior lens), the vasa hyaloidia propria (in the vitreous) and the pupillary membrane (on the anterior surface of the lens in the anterior chamber) (Figure 3A). Regression of the different components of the hyaloid vessel system is temporally well defined. In the rat, regression begins with the vasa hyaloidia propria at about 3 days after birth and is completed between 2 and 4 weeks when the last remnants of the tunica vasculosa are eliminated (Figure 3B–D). In the mouse, the phase of regression is a little more succinct, beginning at about 5 days after birth and being completed by about day 21.

The pattern of apoptosis

Apoptosis has been documented in the vascular endothelial cells (VECs) of the pupillary membrane (PM) in rodents (Lang *et al*, 1994) and in humans (M. Zhu and P. Penfold, personal communication). The PM has proven useful for analysis mainly because it can be removed from the rat eye by dissection and visualized in its entirety (Figure 4A) (Lang *et al*, 1994). Using this technique, combined with TUNEL analysis, the pattern of apoptosis has been assessed over the entire network and has proven illuminating. In humans (M. Zhu and P. Penfold, personal communication) as well as rodents, apoptosis occurs predominantly in two distinct circumstances. In the first, isolated TUNEL-labelled VECs are observed in otherwise normal capillaries and this is referred to as initiating apoptosis (Figure 4B). One also observes capillary segments in which apoptotic cells are distributed along the entire segment (Figure 4C). Since apoptosis and disposal generally takes only a few hours, this observation implies that there is a degree of synchrony to the cell death that occurs in this latter circumstance (referred to as secondary apoptosis) and raises the interesting question of how a synchronous pattern of apoptosis might be orchestrated.

Macrophage involvement

Morphological studies have established that a macrophage-related cell called the hyalocyte is closely associated with the vessels of the PM during its regression. The hyalocyte was identified as a tissue macrophage based on its morphology (Matsuo and Smelser, 1971; Balazs *et al*, 1980) and on marker studies (Lang *et al*, 1994) and was suggested to participate in capillary regression. More recently, genetic ablation studies in transgenic mice have implied that that ocular macrophages are required for regression (Lang and Bishop, 1993). Killing of macrophages with a toxin-expressing transgene led to persistence of the PM. Furthermore, the cells comprising the PM appeared viable suggesting that macrophages were permissive for apoptosis, or were required for its induction.

Based on these considerations, a model explaining programmed capillary regression proposes that regression is begun when macrophages induce apoptosis in some of the endothelial cells that are components of the PM (termed initiating apoptosis) (Figure 5A). The model also suggests that the synchronous pattern of apoptosis occurs as a second step in regression (termed secondary apoptosis) and is a result of VECs being deprived of circulating growth factors when flow within the capillary ceases (Figure 5B).

Consistent with the notion that macrophages may kill is the observation that macrophages are preferentially associated with isolated vascular endothelial cells undergoing apoptosis (Lang *et al*, 1994; Meeson *et al*, 1996). While there are other possible explanations for this preferential association (such as the action of a chemotax-

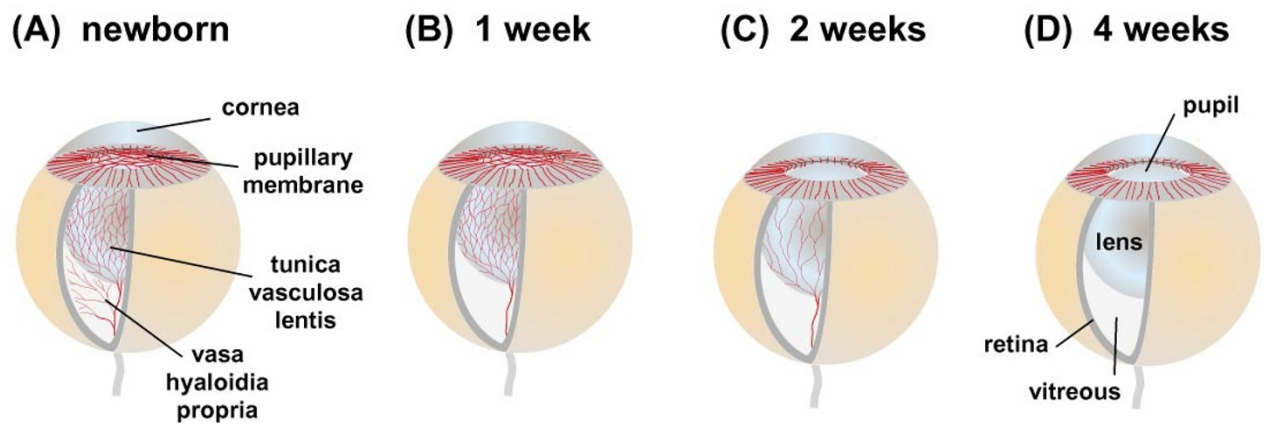

Figure 3 Capillary regression from the postnatal rat eye. (**A**) The newborn rat eye has three capillary networks that will regress over the ensuing 4 weeks. Capillary branches in the vitreous are named the vasa hyaloidia propria and those that surround the lens the tunica vasculosa lentis. The capillaries of the pupillary membrane extend from the iris diaphragm and cover the pupil and anterior pole of the lens. (**B**) By 1 week after birth, the vasa hyaloidia propria has regressed. (**C**) By week 2, the pupillary membrane has completely regressed and the tunica vasculosa lentis partially. (**D**) At 4 weeks, regression of ocular capillaries is complete.

in), it is clear that macrophages require close contact with the target cell if they are to kill (Aliprantis *et al*, 1996).

A number of studies of diverse origin suggest that phagocytes may be capable of killing target cells during normal development and in disease (for review see Aliprantis *et al*, 1996). In the *C. elegans* male, laser ablation of adjacent epidermal cells prevents programmed death of the gonadal linker cell (Sulston and Thompson, 1980). This suggests a programmed death dependent upon a signal from adjacent cells and is, in principle, similar to circumstances where activated macrophages kill tumor cells by inducing apoptosis (van de Loosdrecht *et al*, 1993; Cui *et al*, 1994). Notably, Mantovani and colleagues have shown that activated human macrophages can kill primary vascular endothelial cells (Peri *et al*, 1990) in what may be an *in vitro* correlate of macrophage action during PM regression. The molecular mediators of macrophage cytocidal activity have been characterized *in vitro* and include soluble (Beutler and Cerami, 1992) and membrane bound TNFα (Grell *et al*, 1995), nitric oxide (Cui *et al*, 1994) and reactive oxygen species (Hansson *et al*, 1996).

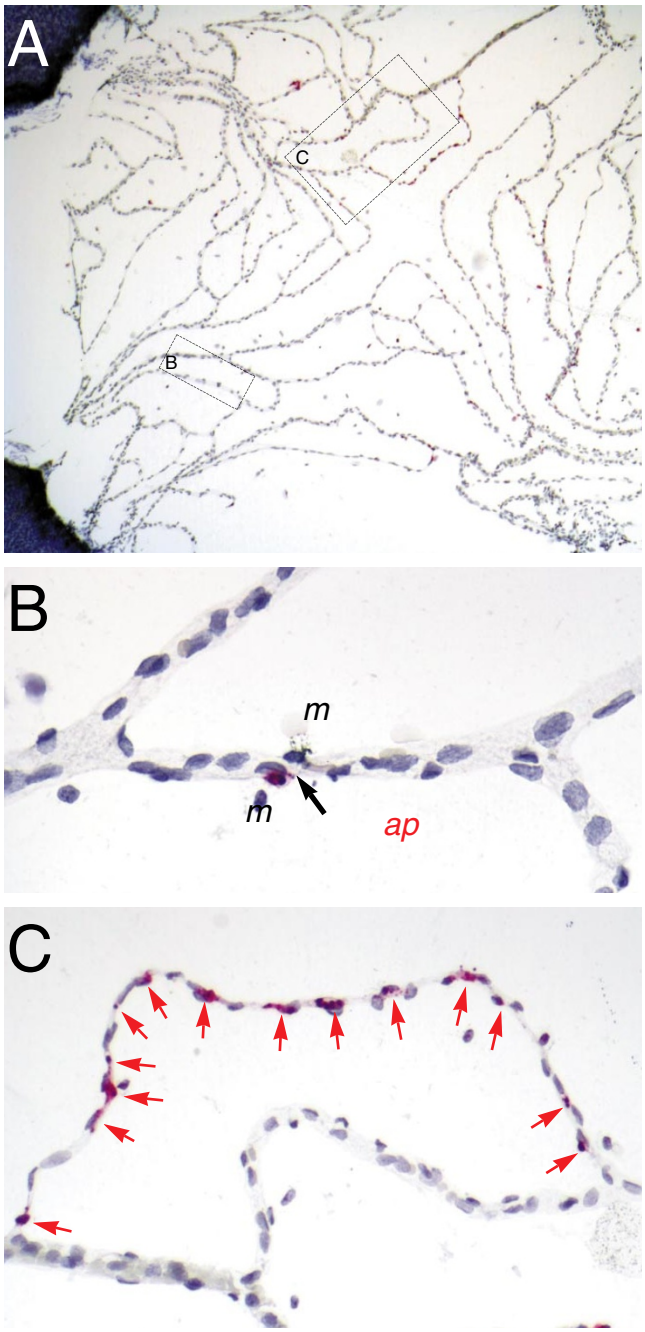

Figure 4 Patterns of apoptosis during programmed capillary regression (**A**) The capillaries of the pupillary membrane at post-conception day 33.5 (50× magnification). The two boxed regions labelled B and C are shown magnified in the lower two panels. (**B**) Initiating apoptosis in a capillary segment is apparent as a single apoptotic endothelial cell in a capillary that otherwise is normal (400× magnification). The TUNEL method has been used to label the apoptotic cell red (arrow). Macrophages are in close attendance (*m*). (**C**) Apoptotic endothelial cells are also seen to apoptose along an entire capillary segment suggesting a degree of synchrony (200× magnification). Apoptoses are labelled by TUNEL (arrows).

Is flow stasis required for programmed capillary regression?

The synchronous pattern of apoptosis observed in segments of the PM during regression (secondary apoptosis, Figure 5B) has led to the proposal that flow stasis may play an important part in regression (Lang *et al*, 1994). Specifically, it has been suggested that VECs will be deprived of a plasma-borne survival factors when flow ceases, and that this may result in the observed segmental pattern of apoptosis.

Vital analysis of capillary regression supports this view with the demonstration that synchronous apoptosis is observed only in capillary segments that show no sign of blood flow (Meeson *et al*, 1996). Conversely, since initiating apoptosis is observed in capillaries that retain vigorous flow (Meeson *et al*, 1996), it is clear that flow stasis and subsequent growth factor deprivation cannot be the explanation for all the apoptosis observed during regression.

While preliminary evidence suggests that neonatal rat plasma does stimulate the survival of the PM in explant assays (A. Meeson and R.A.L., unpublished), confirmation that growth factor deprivation-induced apoptosis is an important component of capillary regression awaits further experimentation. This mechanism is consistent with the emerging view that all cells are dependent on growth factors for their survival *in vivo* (Angeletti *et al*, 1971; Raff, 1992; Chow *et al*, 1995; Campochiaro *et al*, 1996) and in particular with the recent observation that endothelial cell growth factors have survival stimulating activity (Alon *et al*, 1995).

Programmed cell death in ocular immune privilege

The anterior chamber of the eye is a site of immune privilege (Streilein, 1995). This was first established more than a century ago when it was shown that foreign tissue grafted to the eye would remain intact for an extended period of time. It is now understood, contrary to the suggestion that this phenomenon was due to immune isolation (Medawar, 1948), that immune provilege in the anterior eye segment

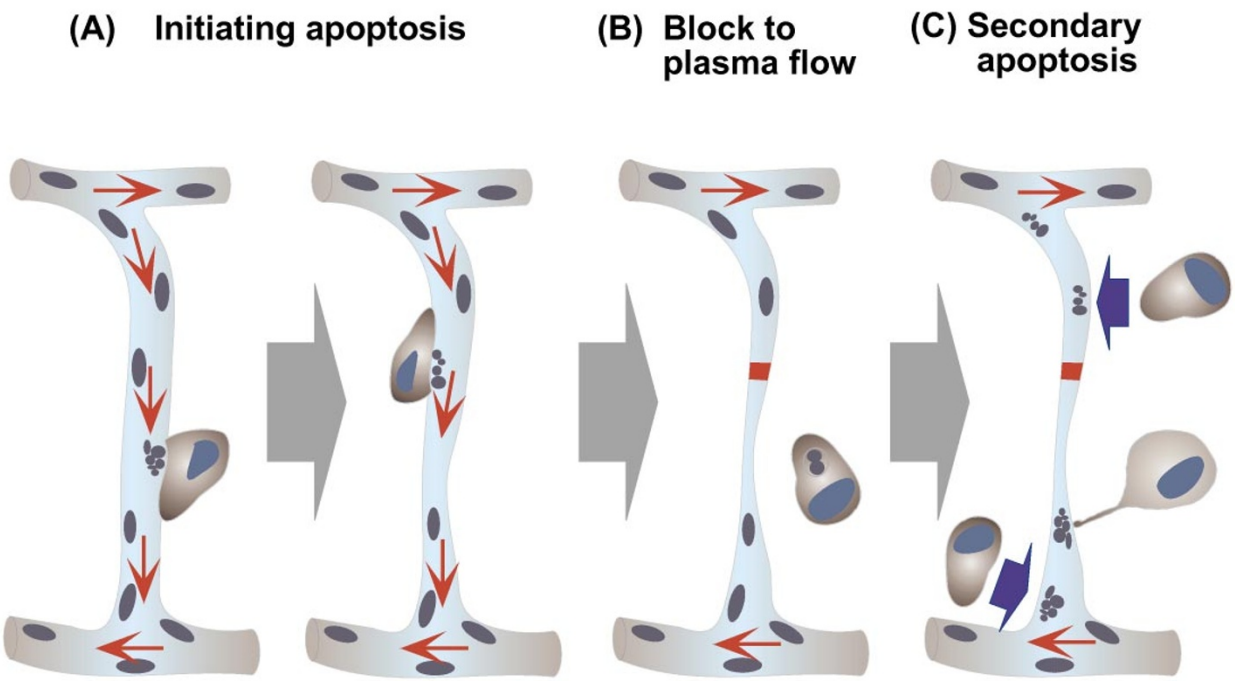

Figure 5 A model for developmentally programmed capillary regression. Current evidence suggests that programmed capillary regression may occur through a two step mechanism. The model suggests that the first apoptoses of vascular endothelial cells are dependent upon the macrophage. This event is termed initiating apoptosis (**A**). We propose that macrophage-mediated VEC death ultimately results in lumen restriction and a block to plasma flow within a capillary segment. Red arrows indicate flow and the red bar, a block to flow (**B**). We suggest that VECs die subsequently with a synchronous pattern because they are denied survival factors present in plasma. This is referred to as secondary apoptosis (**C**). The large blue arrows indicate the likely chemotactic response of macrophages to apoptotic cells.

involves active cooperation of the host and involves a modified immune response. This modified response is referred to as anterior chamber-associated immune deviation or ACAID (Ksander and Streilein, 1994) and is characterized by the migration of antigen presenting cells from the eye to the spleen and the down regulation of T-lymphocyte effector function. ACAID has presumably evolved as a means of minimizing the possibility of vision loss due to inflammation during ocular immune challenge.

The phenomenon of immune privilege resides both in privileged sites and privileged tissues. Privileged tissues are resistant to the action of an immune response through a number of features including surface expression of hyaluronic acid and absent or low levels of MHC (major histocompatibility complex) molecule expression (Ksander and Streilein, 1994). Privileged sites have the property that they can retain a tissue graft from a non-privileged source and achieve this using a combination of blood-tissue barriers, absence of efferent lymphatics, an immunosuppressive environment and inhibitors of complement activation and fixation (Streilein, 1995). Only recently has it become apparent that the special status of privileged tissues and privileged sites may be due to their production of molecules that kill immune effector cells.

The transforming growth factor-β (TGFβ) family of molecules have a wide variety of activities depending upon the target cell. Two of the family members, TGFβ1 and TGFβ2, appear to have a role in immune suppression. It is clear from the systemic inflammatory syndrome in transgenic mice missing the TGFβ1 gene, that TGFβ1 has a general role in suppressing inflammation (Diebold *et al*, 1995). TGFβ2 can be found associated with immune privileged sites including the decidua of the placenta (Clark *et al*, 1988) and the anterior chamber of the eye (Granstein *et al*, 1990). Furthermore, TGFβ2 is known to induce ACAID behaviour in antigen presenting cells (Willbanks and Streilein, 1992).

The anti-inflammatory action of TGFβ may be explained in part by its pro-apoptotic activity towards inflammatory cells. While TGFβ actually suppresses apoptosis in immature CD4 positive lymphocytes (Swain, 1995), in mature cells of the T-lineage (as represented by T-cell lines) TGFβ can directly induce apoptosis (Weller *et al*, 1994). In general however, the action of TGFβ on inflammatory cells including haemopoietic progenitors (Jacobsen *et al*, 1995) mast cells (Mekori and Metcalfe, 1994; Mekori *et al*, 1995) and eosinophils (Alam *et al*, 1994) is to abrogate the effect of survival stimuli. Thus, in the anterior chamber of the eye where high levels of active TGFβ2 are found (Granstein *et al*, 1990), TGFβ will presumably hasten the demise of any inflammatory cell present and aid in the suppression of a potentially damaging response.

Fas ligand (FasL) was recently shown to be expressed in the eye in the corneal endothelium and epithelium, iris, ciliary body and retina (Griffith *et al*, 1995). FasL was originally isolated as the ligand for CD95 Fas, a death-domain (Tartaglia *et al*, 1993) containing cell surface

receptor that mediates the apoptotic elimination of autoreactive T-cells (Nagata and Golstein, 1995). Expression of FasL in the eye raised the interesting possibility that Fas-mediated apoptosis of infiltrating inflammatory cells might be one method for a tissue to acquire privileged status.

Lines of mice mutated for either Fas (designated *lpr*; Watanabe-Fukunaga *et al*, 1992) or FasL (designated *gld*; Takahashi *et al*, 1994) have provided a means of assessing the role of the Fas signalling pathway in ocular immune privilege. Using intra-ocular injection of herpes simplex virus (HSV) to induce an inflammatory response, Griffith *et al* (1995) showed that only in *gld* or *lpr* mice did an inflammatory response occur. In wild type mice, injection of HSV resulted in the rapid apoptosis of infiltrating neutrophils and lymphocytes. Ocular FasL then, together with TGFβ2, may provide a means to control the potentially disasterous consequences of inflammation in the eye and is an example of homeostatic control through an apoptotic signal.

Future perspectives

This, and the accompanying reviews provide a strong indication of the value of the eye as an experimental system for the investigation of mechanisms of programmed cell death. From the definition of inductive interactions in development (Spemann, 1901), to the unfolding of a fascinating tale of evolution with the characterization of the lens crystallins (Piatigorsky, 1993) and the identification of ocular immune privilege and its mediators (Streilein, 1993), the vertebrate eye has yielded many insights into biological processes. It is difficult to imagine that the pace of discovery will slow given the enduring attraction of this organ as an object of investigation.

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