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Review

Role of Müller glia in neuroprotection and regeneration in the retina

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Summary. Glial cells are thought to protect neurons from various neurological insults. When there is injury to retina, Müller cells, which are the predominant glial element in the retina, undergo significant morphological, cellular and molecular changes. Some of these changes reflect Müller cell involvement in protecting the retina from further damage. Müller cells express growth factors, neurotransmitter transporters and antioxidant agents that could have an important role in preventing excitotoxic damage to retinal neurons. Moreover, Müller cells contact to endothelial cells to facilitate the neovascularization process during hypoxic conditions. Finally, recent studies have pointed to a role of Müller cells in retina regeneration after damage, dedifferentiating to progenitor cells and then giving rise to different neuronal cell types. In this article we will review the role of Müller glia in neuroprotection and regeneration after damage in the retina.

Key words: Müller glia, Neuroprotection, Retina, Regeneration, Glutamate, Neurotrophins, Gliosis, Excitotoxicity

Müller cells in the retina

Müller cells constitute the principal glia of the retina and span its entire thickness (Fig. 1A) and their somata lie within the inner nuclear layer, where they may form a distinct median sublayer in some species. Although Müller cells from different species vary considerably in shape, some features are fairly universal. At the level of the outer limiting membrane, Müller cells extend apical microvilli into the subretinal space between the inner segments of photoreceptor cells. In some species, Müller cells also possess a cilium (Ennis and Kunz, 1986). The length and number of microvilli vary between species, probably in inverse relation to the degree of retinal vascularization (Uga and Smelser, 1973). Apicolaterally, Müller cells are connected to their neighbouring Müller and photoreceptor cells by specialised junctions to form the outer limiting membrane. In most vertebrate species studied these junctions are zonulae adhaerens or tight junctions (Reichenbach and Robinson, 1995). However, amphibian Müller cells are connected by intermediate junctions, and in frogs and toads by gap junctions (Uga and Smelser, 1973) that permit extensive electrical coupling (Attwell et al., 1985). Although human Müller cells are reported to express gap junctions in the inner retina (Reale et al., 1978), neither gap nor tight junctions have been demonstrated in the apicolateral membrane of mammalian Müller cells in situ. Gap junctions between Müller cells and astrocytes have been demonstrated in the rabbit retina with dye-coupling (Robinson, 1992; Robinson et al., 1992), but Müller cells are never dye-coupled to each other.

Müller cells send side branches into the two plexiform layers of the retina where they form sheaths around neuronal processes and synapses, particularly around the photoreceptor pedicles in the outer plexiform layer (Reichenbach et al., 1989). In the nuclear layers, the lamellar processes of Müller cells form structures that envelop the cell bodies of neuronal cells (Hama et al., 1978; Reichenbach et al., 1988, 1989; Dreher et al., 1992). Müller cells extend smooth and sometimes rather long processes through the nerve fiber layer ending in a basal end-foot, which lies adjacent to the inner limiting membrane. This membrane is a basal lamina, at least partly produced by Müller cells. On the other hand, Müller cells are involved in the structural organization of the blood-retina barrier (Reichenbach and Robinson, 1995). Blood capillaries are ensheathed by Müller cell processes, who act as a communicating system for metabolic exchange between vasculature and neurons in much the same manner as postulated for brain astrocytes. However, the close physical association between Müller cell processes and the retinal vessels still leaves open questions as to whether the Müller cell per se is an essential component of the blood-retina barrier, and whether it is capable of influencing its permeability characteristics (Sarthy and Ripps, 2001).

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Considering their strategic location, Müller cells are in a position to influence and be influenced by neuronal activity. In this sense, it has been shown that the Müller cells are the major supportive glia for neurons in the adult retina and perform many of the functions performed by oligodendrocytes, astrocytes and ependymal cells in other regions of the central nervous system (Reichenbach and Wohlrab, 1986; Holländer et al., 1991; Bignami, 1995; Newman and Reichenbach, 1996).

There is little variation in the number of ganglion cells per Müller cells. Likewise, the number of neurons in the inner nuclear layer and even the number of cone photoreceptors per Müller cells seem to be fairly similar among the species. In contrast, the number of rods per Müller cell may vary over a wide range. This relationship has led to a hypothesis describing the generation of retinal cells. In the mammalian retina, an early proliferative phase produces several types of "primary" neurons. In a second proliferative phase, columnar clones are generated each from a common progenitor; such a clone consists of one Müller cell and a defined number of rod photoreceptors, bipolar cells, and amacrine cells. These neurons become ensheathed by lateral cytoplasmic excrescencies of the Müller cell, and seem to form a metabolic and functional unit together with this cell. The idea that each Müller cell subserves many of the metabolic, ionic and extracellular buffering requirements of those neurons with which it shares a common progenitor is almost accepted (Reichenbach et al., 1993). A similar phenomenon has been observed in the avian optic tectum (Gray and Sanes, 1991, 1992); progenitor cells resembling radial glial cells produce cell clones that consist of one radial glial cell and up to 15 neuronal cells. The latter migrate along the radial glial process to reach the tectal plate (Reichenbach and Robinson, 1995).

Like other glial cells, Müller cells express a wide variety of voltage-gated ion channels and also express many types of neurotransmitter receptors, including a gamma-aminobutyric acid (GABA) receptor and several types of glutamate receptors. It is known that Müller cells have the capacity to modulate extracellular fluid bathing in retinal cells. The concentrations of neurotransmitters and potassium, for example, are regulated by glial cell homeostatic mechanisms. Müller cells possess high-affinity uptake carriers for many transmitters and are believed to regulate extracellular transmitter levels in the retina (Sarthy and Ripps, 2001).

Müller cells and neuroprotection

Protection against excitotoxic damage

Glutamate transporters

Glutamate, an excitatory amino acid neurotransmitter, can have potent neurotoxic activity if its extracellular concentration becomes elevated in the central nervous system, including the retina (Lucas and Newhouse, 1957; Choi, 1987; Choi et al., 1987; Rothman and Olney, 1987; Frandsen and Schousboe, 1993; Heidinger et al., 1997). Hence, excessive glutamate is thought to be responsible for a variety of acute neurological insults, including ischemia and anoxia, hypoglycemia, trauma, and several chronic neurodegenerative diseases (Choi, 1988; Meldrum and Garthwaite, 1990).

The primary glutamate transporter expressed by retinal astrocytes and Müller cells is the L-glutamate/L-aspartate transporter (GLAST) (Otori et al., 1994), which has been postulated to contribute to the clearance of glutamate and to protect retinal ganglion cells from glutamate neurotoxicity (Derouiche and Rauen, 1995; Kitano et al., 1996; Lehre et al., 1997; Matsui et al., 1999).

The first evidence about the excitotoxic effect of a high level of glutamate was given by Lucas and Newhouse (1957), who showed that subcutaneous injections of sodium L-glutamate in adult and neonatal mice resulted in severe destruction of ganglion cells and partial loss of cells in the inner nuclear layer. Other studies confirmed the excitotoxic effect of glutamate and its relative selectivity for neurons of the inner retina when it was administered subcutaneously (Cohen, 1967) or intravitreally (Sisk and Kuwabara, 1985).

Various studies have shown that Müller cells can protect against the excitotoxic effects of glutamate in the whole retina and increase survival of ganglion cells in culture (Kitano et al., 1996; Heidinger et al., 1999; Izumi et al., 1999; Kawasaki et al., 2000). This protective effect seems to involve uptake of glutamate from the medium through the GLAST transporter (Kawasaki et al., 2000).

Changes in the expression of GLAST has been demonstrated during some ocular diseases. Thus, an increase in GLAST activity has been described during glaucoma in monkeys (Carter-Dawson et al., 2002). A small elevation in the concentration of glutamate when maintained for long periods of time is toxic to retinal ganglion cells (Vorwerk et al., 1996). This relatively small (micromolar) rise in glutamate is typically seen in the vitreous of human and monkey eyes with glaucoma (Dreyer et al., 1996) and in dogs with naturally occurring glaucoma (Brooks et al., 1997). However, a recent study has not found an increase in vitreal glutamate concentration in glaucomatous eyes from monkeys (Carter-Dawson et al., 2002). This finding does not eliminate the role for glutamate excitotoxic damage in glaucoma since these authors have described an increase in glutamine, glutathione and GLAST activity. Increases in glutamine, glutathione, and GLAST content in glaucomatous monkey eyes indicate an elevation and an enhancement in glutamate transport and metabolism, but the possibility of excitotoxic damage to ganglion cells is not eliminated (Carter-Dawson et al., 2002). Increases in GLAST mRNA following transient retinal ischemia has also been found (Otori et al., 1994). In

contrast, no changes in the expression of GLAST are observed after occlusion of the central retina artery (Barnett et al., 2001), but a decrease in glutamate transporter activity has been described in the diabetic retina (Li and Puro, 2002). A potentially critical feature of GLAST is the presence of redox-sensing elements, which regulate this transporter through thiol-disulfide redox interconversion (Trotti et al., 1997, 1998). Oxidative stress occurs in the retina early in the course of diabetes (Kowluru et al., 1996, 2001; Ellis et al., 2000), and it has been shown that GLAST activity is decreased and glutamate levels are elevated in the diabetic retina (Kowluru et al., 2001).

It is not clear whether the protective effect of Müller cells against glutamate neurotoxicity is dependent on glial cell-retinal ganglion cell contact. Thus, Kawasaki et al. (2000) have shown that the protective effect of Müller cells on retinal ganglion cell survival in the presence of a high level of glutamate does not depend on cell contact. In contrast, other reports have suggested that this neuroprotective effect needs cell-cell contact (Heidinger et al., 1999). The protective effect of Müller cells also seems to depend on confluence of glial cells, since Kashiwagi et al. (2001) found that confluent retinal glial cells are able to take up and metabolize excessive glutamate, whereas non-confluent retinal Müller cells do not have this effect (Kashiwagi et al., 2001).

Glutamine synthetase

In physiological conditions, after its release from neurons, glutamate is quickly taken up by glial cells and amidated to form the non-neuroactive compound glutamine (Curtis and Watkins, 1969; Hertz et al., 1992). This amidation is catalyzed by the enzyme glutamine synthetase, which is confined exclusively to the Müller glial cells in the retina (Riepe and Norenberg, 1977; Linser et al., 1984). It has been demonstrated that the temporal and spatial pattern of glutamine synthetase expression in the Müller glial cells is not related to Müller cell differentiation but to a synaptogenesis process in the outer plexiform layer, at least in chicken retina (Prada et al., 1998).

Gorovits et al. (1997) demonstrated that glutamine synthetase is directly involved in neuroprotection by showing that chick retinas treated with cortisol in order to induce high levels of glutamine synthetase are more resistant to glutamate-induced damage than retinas expressing low levels of the enzyme. Besides, by using mixed cultures of neuronal cells seeded onto glial cells it has been show that glutamate induces an increase in glutamine synthetase activity in mature and also immature mixed retinal cell cultures, although only adult glia induce protection (Heidinger et al., 1999). Moreover, increases in glutamine synthetase activity only occurs in mixed neuronal-glial cultures, indicating that neuron-glial cell contacts are required for the regulation of the enzyme (Vardimon et al., 1988; Mearow et al., 1990; Prada et al., 1998; Heidinger et al., 1999). Therefore, all these works point to the fact that excitatory amino acids by themselves do not up-regulate glial glutamine synthetase expression but that neuron-glial cell contacts are required in the regulation of this enzyme. The existence of a diffusible factor released by neurons damaged under excitotoxic conditions capable of activating glutamine synthetase activity is possible.

A decrease in activity of glutamine synthetase has been observed in pathologies such as diabetes (Lieth et al., 2000). However, it has been shown that in an experimental model of glaucoma in monkey, glutamine immunolabeling is greater in Müller cells of glaucomatous eyes, suggesting that Müller cell function in the glutamate-glutamine cycle continues in glaucoma (Carter-Dawson et al., 1998). Finally, no changes in glutamine synthetase expression in Müller cells are observed after optic nerve crush in rats. In the last case, the enzyme shows a transient shift in its cellular distribution and translocates from the cell body to the inner and outer Müller processes and particularly to the basal endfeet located in the ganglion cell layer (Chen and Weber, 2002).

The activity of glutamine synthetase can be down-regulated by a trophic factor since it has been observed that basic fibroblast growth factor (bFGF), in addition to its reported neuroprotective effect, may produce a direct down-regulation of the enzyme and therefore exacerbate glutamate-mediated neurotoxicity (Kruchkova et al., 2001).

Gluthatione

Reactive oxygen species are generated in the retina under various conditions such as anoxia and ischemia. One of the crucial substances protecting the retina against reactive oxygen species is glutathione, a tripeptide constituted of glutamate, cysteine and glycine. Glutathione in the retina of the rabbit has been located in Müller cells, suggesting that glia play a critical role in regulating the content of potentially damaging oxidative species in the retina (Pow and Crook, 1995). The low mithochondrial content of gamma-glutamylcysteine (the direct precursor of glutathione) is consistent with biochemical data indicating that glutathione is synthesized extramitochondrially and transported into the mitochondrial matrix of Müller cells (Huster et al., 1998). Intracellular glutamate concentration of the Müller cells is determined by the extracellular glutamate concentration and the velocity of the sodium/glutamate uptake (Reichelt et al., 1997).

Physiological concentrations of glutathione can protect Müller cells from oxidative injury. Both Na^+-dependent and Na^+-independent transport systems for glutathione exist in Müller cells, and the Na^+-dependent glutathione transporter may be involved in the protective role of glutathione (Kannan et al., 1999).

On the other hand, a transfer of glutathione from Müller cells to neurons has been observed under ischemic conditions in rat retina (Schutte and Werner,

1998). During impaired glutamate uptake, as is observed during total ischemia, a glutamate deficiency occurs in Müller cells. In this condition, this amino acid is preferentially delivered to the glutamate-glutamine pathway at the expense of glutathione. This mechanism may contribute to the finding that total ischemia causes a depletion in glial glutathione. The ischemia-induced lack of glutathione is particularly fatal considering the increased production of reactive oxygen species under this condition (Huster et al., 2000).

Neurotrophic factors

The low affinity neurotrophin receptor, p75, is expressed in Müller glia in retinas from different species (Fig. 1B,C). This receptor seems to be implicated in neuroprotection in the retina, since an up-regulation of p75 on Müller cells is observed in pathologies such as diabetes (Hammes et al., 1995) and after ischemia and reperfusion in rats (Tomita et al., 1998; Vecino et al., 1998) (Fig. 2A,B). In physiological conditions, the low affinity receptor, p75, is located in the extensions of the Müller cells to form three horizontal laminae of immunoreactivity in the inner plexiform layer and one lamina within the outer plexiform layer in the rat retina. Moreover, the immunoreactivity is also associated with the radial processes of the Müller cells running from the inner to the outer parts of the retina. After ischemia and reperfusion a change in the intensity and distribution of the p75 immunoreactivity is observed. The three distinct layers of immunoreactivity originating from the extensions of the Müller cells and present in the inner plexiform layer in control retina (Fig. 2A) are no longer present but rather appear as a single thick layer (Fig. 2B). The immunostaining associated with the radial branches of the Müller cells also increase but in a disorganized manner. Increase in the p75 expression in Müller cells after ischemia-reperfusion suggests that this receptor may be involved in the enhancement of Müller cell response to neurotrophins (Vecino et al., 1998).

It has been demonstrated that release of neurotrophic factors from glial cells increases the long-term survival of developing retinal ganglion cells in culture (Meyer-Franke et al., 1995). Survival of retinal ganglion cells in the Müller-conditioned media seems to decline as the differentiation of neurons proceeds, since Müller cells in

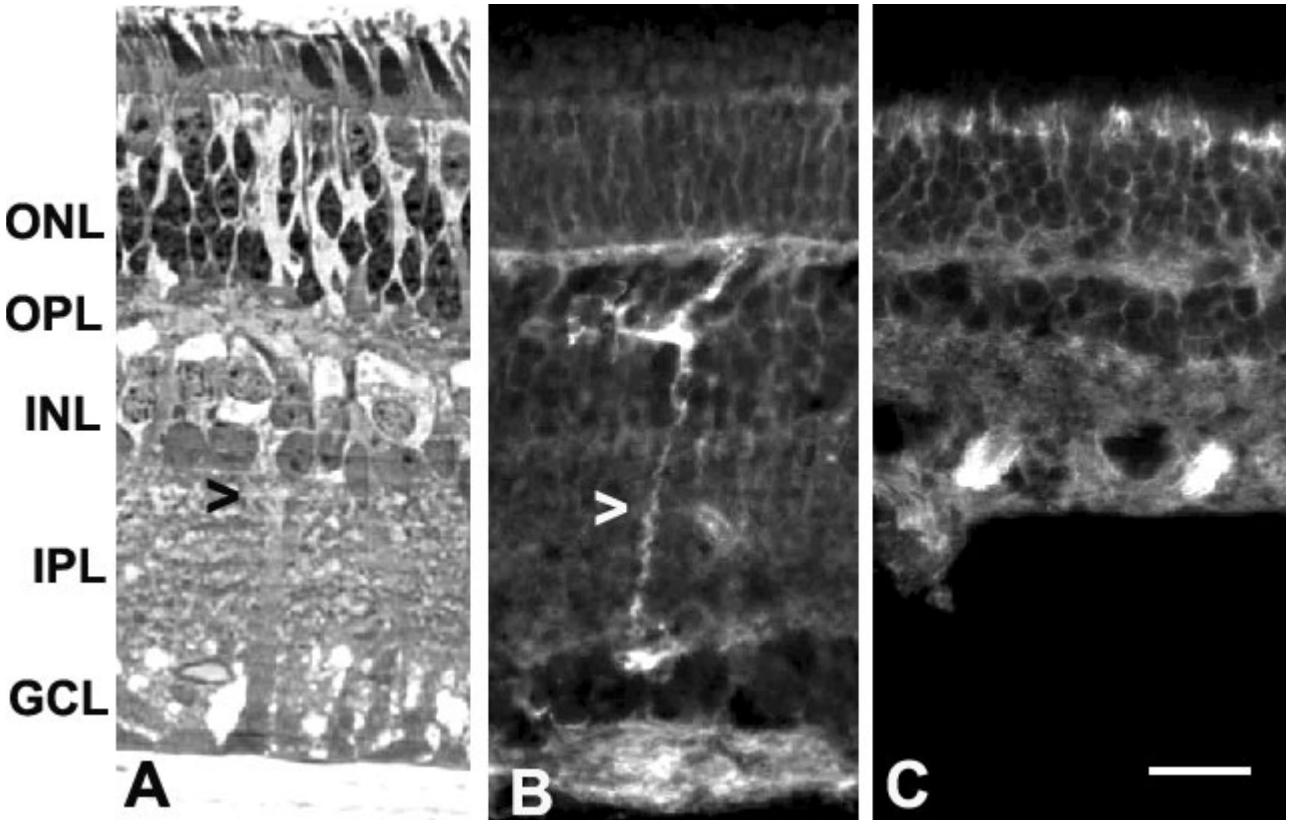

Fig. 1. A. Photomicrograph of a semi-thin section from pig retina stained with toludine blue. Müller cells (open arrowehead) are radially oriented cells that transverse the retina from its inner border to the distal end of the outer nuclear layer. Along their course, Múller cells extend branches that interdigitate with every class of retinal neurons, with other types of glia and with blood vessels. **B.** Photomicrographs of low affinity NGF-receptor, p75, localization in normal porcine retina. The location of the p75 receptor within the Müller cells and their process is observed (open arrowhead). **C.** Distribution of p75 receptor in Müller cells from human retina. Scale bar: 50 μm.

culture increase the survival of retinal ganglion cells from rats at birth, but not on retinal ganglion cells from 6-day postnatal rats (Raju and Bennett, 1986). However, a recent work has demonstrated that Müller cells increase the survival and enhance the neuritogenesis of retinal ganglion cells in cultures from adult pig retinas (García et al., 2002). The protective effect observed in retinal ganglion cells co-cultured with confluent Müller

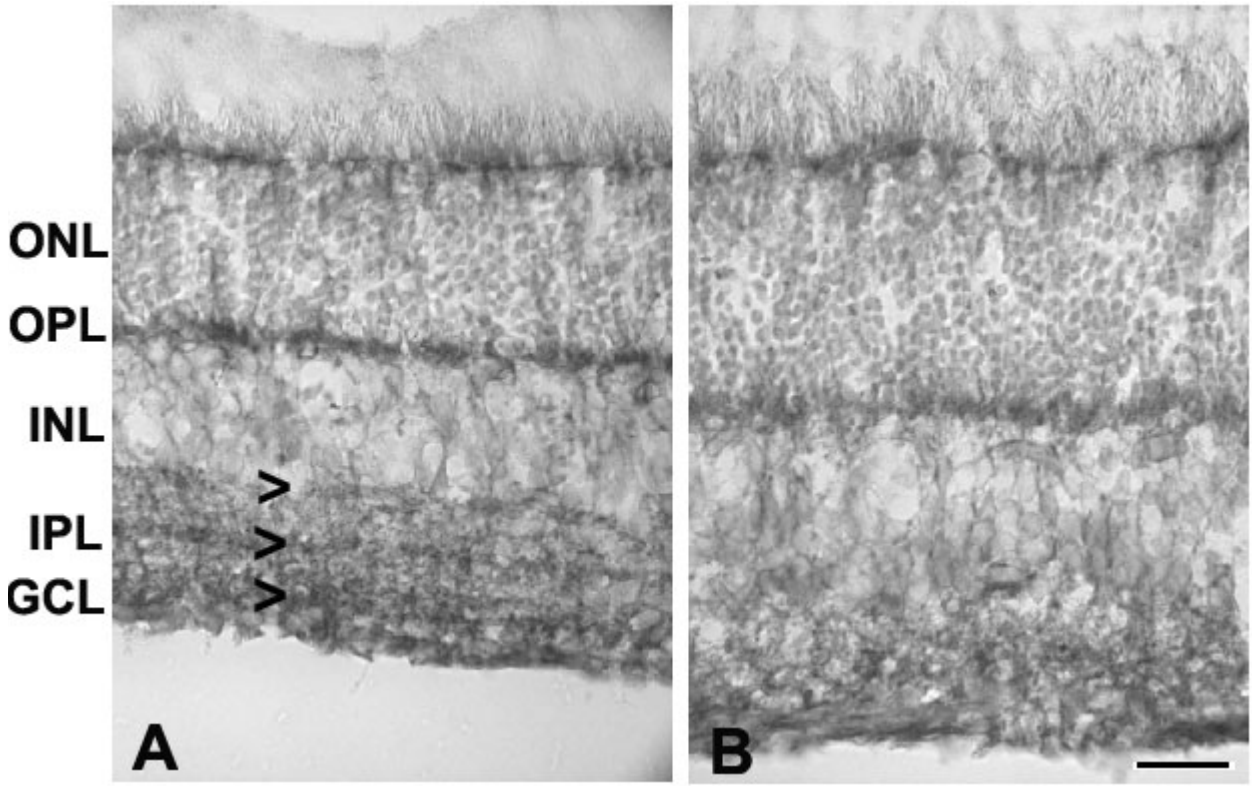

Fig. 2. Distribution of p75 receptor within the Müller cells in control rat retina and after ischemia reperfusion. **A.** Control retina. **B.** Retina after 1 hour of ischemia followed by 2 hours of reperfusion. Note that most Müller cells are immunostained. In control retina, there are three laminae of immunoreactivity (open arrowheads) whereas in damaged retina there is a thick band of immunoreactivity occupying most of the inner plexiform layer. Scale bar: 50 μm.

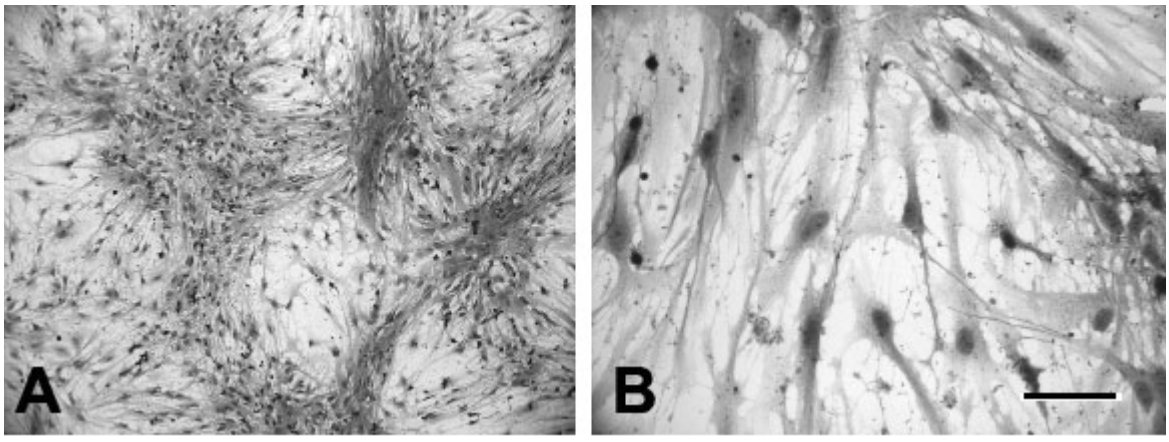

Fig. 3. Confluent Müller glia from porcine retina after six days in culture. Staining was performed with toluidine blue. **A.** Photomicrograph of cultured Müller glia at low magnifications. Scale bar: 200 μm. **B.** High magnification micrograph showing the morphology of Müller glia on culture. This morphology is similar to that observed in the retina in vivo. Scale bar: 50 μm.

cells seems not to be mediated only by substrate but also by factor(s) secreted by retinal Müller glia (García et al., 2002). Other authors have found that Müller cells have a neurite-promoting effect on retinal ganglion cells only when there is a direct cell-to-cell contact, whereas soluble factors released by Müller cells do not induce significant neurite outgrowth in retinal ganglion cells (Raju and Bennet, 1986). These differences could be due to the age of the Müller cell cultures; thus, cultures of Müller glia used by Garcia et al. were 6 days old and in these conditions Müller cells are confluent and exhibit similar molecular characteristics to those seen *in vivo* (Fig. 3A,B). However, it has been demonstrated by proteomic technique, that Müller cells in culture change their protein expression dramatically and when the cultures are older than seven days, the molecular characteristics of the cells are no longer the same as in recently dissociated cells (Ueffing, personal communication).

Neurotrophic factors from glial cells diminish the retinal ganglion cell degeneration after optic nerve damage in rat (Yan et al., 1999). In other species, like fish, Müller cells seem to play an important role during the optic nerve regeneration after damage. Thus, in tench retina the BDNF is expressed mainly in the Müller cells and in cells in the ganglion cell layer, and after optic nerve crush an increase in BDNF expression in Müller glia is observed (Caminos et al., 1999) Considering the effects of BDNF on ganglion cells and the permanent production of BDNF by fish, Müller cells may be important in the capacity of retinal ganglion cells to regenerate their axons in the tench. (Fig. 4A, B).

Müller cells have also been implicated in the determination of photoreceptor survival. It has been demonstrated that blockage of p75 prevents bFGF reduction mediated by NGF, resulting in an increase of both structural and functional photoreceptor survival in vivo (Harada et al., 2000). Moreover BDNF, ciliary neurotrophic factor (CNTF) and fibroblast growth factor FGF2 may exert their effects on photoreceptors by acting indirectly through activation of Müller cells (Wahlin et al., 2000, 2001). Finally, it has been demonstrated that the α_2- adrenergic agonists have specific and selective effects on the retina to induce expression of basic fibroblast growth factor and to protect photoreceptors. When the α_2-adrenergic agonists xylazine and clonidine were administered systemically, an increase in extracellular signals regulated kinase (ERK) phosphorylation is observed in Müller cells. This datum suggests that activation of ERKs in Müller cells is probably one of the early events that result in photoreceptor protection and imply a role for Müller

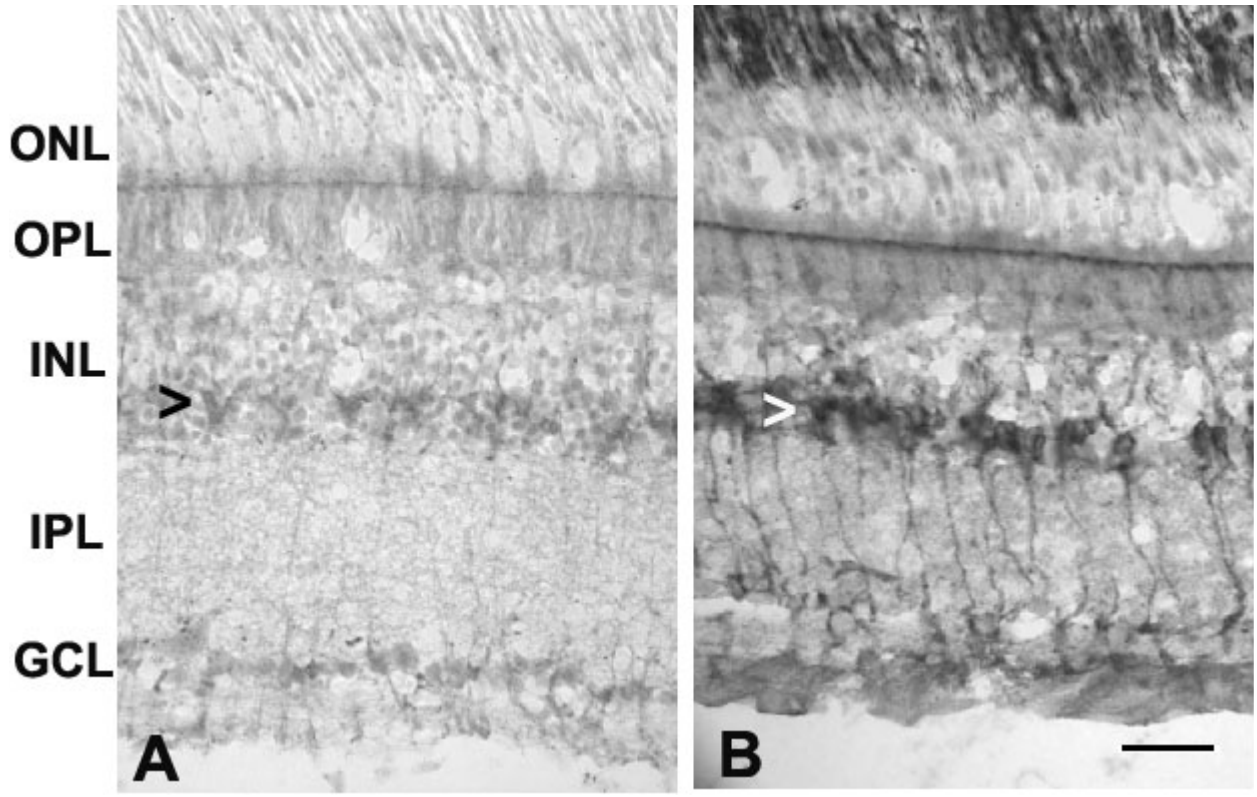

Fig. 4. A. Photomicrograph of brain-derived neurotrophic factor immunolocalization in a section from normal tench retina. Immunoreactivity is observed in Müller cell bodies (open arrowhead) and in process from the inner plexiform layer to the external limiting membrane. **B.** Section from tench retina four days after optic nerve crush. BDNF-immunostaining is markedly increased in Müller glia after the damage. Scale bar: 50 μm.

cells in α_2-adrenergic agonist-induced photoreceptor protection (Peng et al., 1998).

Müller cells are also involved in bipolar cell neuroprotection since it has been demonstrated that the inhibition of p75 receptor-binding eliminates BDNF-promoted bipolar cell survival, but has no effect on FGF2-mediated survival. Interestingly, p75 is expressed by Müller cells but not by bipolar cells, providing for the possibility that BDNF might induce Müller cells to produce a secondary factor, perhaps FGF2, which directly rescues bipolar cells. This hypothesis is supported since an antibody that neutralizes FGF2 attenuates the trophic effects of BDNF. Therefore, BDNF increases production or release of FGF2 by binding to p75 on Müller cells (Wexler et al., 1998).

Müller cells and immunity

In the nervous system, astrocytes, oligodendrocytes and microglia secrete cytokines and have the capacity to respond to them and present antigens to T lymphocytes (Benveniste, 1993). Antigen-presenting cells (APC) in the eye may be classified into bone marrow-derived cells such as uveal dendritic cells and retinal microglia, or nonbone marrow-derived cells represented by retinal pigment epithelium, Müller cells, and vascular endothelial cells (Sarthy and Ripps, 2001). Histopathological studies have shown that Müller cells are involved in the immune response during pathologies like subretinal fibrosis and uveitis syndrome (Kim et al., 1987).

Experimental autoimmune uveoretinitis induced by retinal antigens is a CD4+ lymphocyte-mediated disease. Generation of autoreactive CD4+ cells requires the processing and presentation of autoantigen by antigen-presenting cells in combination with MHC (major histocompatibility complex) class II antigen. It has been demonstrated that several ocular cells express class II antigens during inflammation, while other cells such as Müller cells inhibit antigen presentation in vitro (Forrester et al., 1990).

Products of inflammatory cells secreted in the course of intraocular immune processes induce the expression of surface antigens by Müller cells (Roberge et al., 1985). Expression of MHC class II antigen in equine recurrent uveitis is observed in proliferating Müller cells (Romeike et al., 1998). Moreover, Müller cells can be induced to express MHC class II determinants in culture when exposed to supernatant from activated lymphocytes (Roberge et al., 1988). Interactions between the retinal glial Müller cells and T-lymphocytes can be diverse and even opposite in different conditions, suggesting that Müller cells could play a determining role in the course of immune reactions at the level of the neuro-retina. This dual effect of Müller cells has been observed in vitro on autoimmune T-helper lymphocytes. In a coculture system, Müller cells have a primary inhibitory effect on the proliferation of T lymphocytes while in conditions where their inhibitory action is suppressed Müller cells stimulate T-cells (Chan et al., 1991).

In vivo, retinal diode laser photocoagulation, used frequently in certain retinal pathologies, stimulates a wound healing response in the outer retina and the choroid, producing a cellular infiltrate including macrophages and activated CD4T cells. In this situation, Müller cells express MHC Class II antigen and the intercellular adhesion molecule, ICAM-1, an accessory molecule required for an efficient presentation of antigen to T cells (Richardson et al., 1996). In addition to this role as immunoregulatory cells, Müller cells may participate in healing and scar formation following experimental allergic uveitis (Rao et al., 1986). These glial cells are involved in the formation of the outer limiting membrane, which can potentially act as an anatomical barrier and thus prevent entry of circulating lymphocytes into the retina when the blood-retina barrier is comprised. If antigen-specific T cells escape into the retina, Müller cells may become activated and suppress T-helper cell proliferation by direct cell-cell contact (Caspi and Roberge, 1989).

The potential role of Müller cells in the immune response is mainly based on experimental evidence obtained using in vitro cell culture systems. The role of Müller cells *in vivo* is not clear. Ishimoto et al. (1999) have demonstrated that bone marrow-derived antigen-presenting cells are those likely to be involved in initiating uveoretinitis. Moreover, analysis of presence of antigen-presenting cells in the retina in an experimental intraocular inflammation in rats shows that vascular endothelia, retinal pigment epithelia, Müller cells, and astrocytes do not express either class II molecules or macrophage markers. Only a subpopulation of retinal microglia, those derived from bone marrow, can express MHC class II (Zhang et al., 1997).

Müller cells and phagocytosis

Phagocytosis of exogenous particles, cell debris and hemorrhagic products may be an important mechanism in the tissue repair after injury (Sarthy and Ripps, 2001). Friedenwald and Chan (1932) reported the first evidence of phagocytic activity by Müller cells after the injection of a suspension of melanin granules into the vitreous of albino rabbits. Subsequently, it was shown that materials such as erythrocyte debris and subretinal hemorrhage are phagocytized by Müller cells (Koshibu, 1978; Miller et al., 1986; Mano and Puro, 1990).

In the developing retina, fragmenting DNA is principally phagocytosed by microglia and Müller cells. In postnatal retinas, microglia are the predominant phagocytes for cells dying in the ganglion cell layer and inner nuclear layer, whereas Müller cells appear to be able to phagocyte cells dying in any retinal layer and, since microglia do not normally enter the outer nuclear layer, they may be important for the phagocytosis of dying photoreceptors (Egensperger et al., 1996). In some retinal diseases and following transplantation of retinal

pigment epithelium, melanin granules are liberated to the subretinal space. Recently it has been found that implantation of melanin granules in the subretinal space of albino rabbits may induce a phagocytic cellular response in macrophages and Müller cells (Crafoord et al., 2000).

Mano and Puro (1990) found that human Müller cell cultures from postmortem eyes were able to phagocytize retinal fragments as well as latex beads through a mechanism similar to that observed in macrophages. Rabbit retinal Müller cells in vitro also show an intense phagocytosis of latex beads (Stolzenburg et al., 1992).

However, in other species like fish, Wagner and Raymond (1991) did not find phagocytic activity of Müller glia cells in vivo. Müller glial cells from goldfish are phagocytic for latex beads when they are isolated in cell cultures but not when they are in contact with other cells within the retina (Wagner and Raymond, 1991).

Müller cells and reactive gliosis

Reactive gliosis refers to cytological changes, such as hypertrophy, enlargement of the cell body, swelling of cellular processes and increase in cell number, observed in glial cells after nerve damage (Landis, 1994; Ridet et al., 1997). Müller cell bodies get a rounded shape and Müller cell endfeet become enlarged. Moreover, fine processes of Müller cells are retracted or lost and the distal processes become more tubular. A displacement of Müller cell nuclei from the inner nuclear layer to the outer nuclear layer is also observed as a consequence of retinal detachment. Other changes observed during reactive gliosis of Müller cells include the increase in the number of glycogen granules, Golgi cisternae and rough endoplasmic reticulum (Sarthy and Ripps, 2001).

Virtually every disease of the retina is associated with a reactive Müller cell gliosis, which may either support the survival of retinal neurons or accelerate the progress of neuronal degeneration (Bringmann and Reichenbach, 2001). Müller cell proliferation has been reported in proliferative diabetic retinopathy and massive retinal gliosis (Nork et al., 1986, 1987). Although Müller cells are postmitotic in the mature retina, they retain the capability to undergo mitotic activity under pathological conditions. Following neuronal damage, Müller cells undergo proliferation and changes in gene expression. Downregulation of tumor-suppressor protein p27kip1 and re-entry into the cell cycle occurs after retinal injury. Thereafter, Müller glial cells up-regulate genes which are typical of gliosis and down-regulate cyclin D3 in concert with an exit from mitosis (Dyer and Cepko, 2000).

Besides genes involved in cell cycle regulation, alterations in other gene expression has been observed in Müller cells in response to injury. Proteins such as glial fibrillar acidic protein (GFAP), GLAST transporter and CNTF are up-regulated under pathological conditions whereas other proteins such the glutamine synthetase seem to be down-regulated (Linser and Moscona, 1979; Sarthy, 1991; Otori et al., 1994; Wen et al., 1995).

During Müller cell gliosis, an increase in intermediate filament content is observed (Fisher and Anderson, 1994). The major intermediate filament expressed by reactive Müller cells is GFAP, this filament is expressed at a low level or is not detectable in mammalian Müller cells in physiological conditions. The Müller cells up-regulate their GFAP expression following a wide variety of pathological states in retina including age-related retinal degeneration (Diloreto et al., 1995), retinal ischemia (Larsen and Osborne, 1996; Kim et al., 1998), inherited retinal dystrophy (Roque and Caldwell, 1990), retinal hyperoxia (Penn et al., 1998), induced retinal degeneration (Eisenfeld et al., 1984), glaucoma (Tanihara et al., 1997) and diabetes (Lieth et al., 1998; Barber et al., 2000; Rungger-Brandle et al., 2000; Li et al., 2002). An increase in GFAP expression is due to transcriptional activation of the GFAP gene in Müller cells (Sarthy and Fu, 1989). Although it has been suggested that one possible factor that induces GFAP expression could be the loss of cell-cell contact between retinal neurons and Müller cells, this hypothesis is not clear (Lewis et al., 1988; Hicks and Courtois, 1990). In this sense, it has been demonstrated that loss of neighboring neurons leads to major alterations of both the shape and metabolism of Müller cells in rats with inherited retinal dystrophy (Hartig et al., 1995). However, others studies suggest that neuronal loss does not appear to be a prerequisite for GFAP induction (Fitzgerald et al., 1990; Osborne et al., 1991). Some reports have found that an increased rate of neuronal loss was paralleled by the presence of greater numbers of more active Müller cells in a model of glaucoma in rats (Wang et al., 2000). Moreover, Eisenfeld et al. (1984) and Diloreto et al. (1995) showed that Müller cell changes accompanied photoreceptor degeneration in time and location in the rat retina. On the other hand, GFAP induction could be mediated by the action of extracellular, diffusible substances since it has been shown that a focal damage to retina produces a GFAP accumulation in Müller cells remote from the site of injury (Bignami and Dahl, 1979; Humphrey et al., 1993; Verderber et al., 1995). There is some evidence that growth factors, like bFGF, and cytokines like CNTF are signaling molecules involved in GFAP induction (Lewis et al., 1992; Sarthy et al., 1997). Free radicals could also be involved in GFAP induction since it has been found that free radical scavengers inhibit the expression of GFAP in degeneration of photoreceptors (Grosche et al., 1997). The cellular source of the extracellular signaling could be neurons undergoing degeneration (Hageman et al., 1991) or activated macrophages that invade the retina (Cuthbertson et al., 1990).

Although the specific function of reactive gliosis is not clear, it has been postulated that it is involved in phagocytosis to clear the neuron degeneration products (Pearson et al., 1993). Besides, glial cells undergoing reactive gliosis up-regulate production of cytokines and neurotrophic factors which could be important for

neuronal survival. Another possible function of reactive gliosis is the restoring of the blood-brain barrier by scar formation. The increase in GFAP expression may be related to the membrane remodeling events at the outer limiting layer or may be involved in stabilizing Müller cell contacts with the retinal pigmented epithelium or Bruch's membrane in pathologies such as retinitis pigmentosa or atrophic macular lesions. Finally, by analogy with other intermediate filaments, GFAP could act providing added mechanical stability to retina. When rabbits are dosified with sodium iodate, large expanses of retinal pigment epithelium and photoreceptors are destroyed and a subretinal scar, consisting mainly of the ascending processes of Müller cells, replaces them (Korte et al., 1992). Experimental retinal holes in rabbit retina are filled with tissue consisting of Müller cell processes playing a role in retinal hole closure (Hara et al., 2000).

Müller cells and regeneration

The vertebrate retina is derived from paired evaginations from the neural tube in embryonic development and it is initially produced by progenitor cells similar to those that generate the neurons and glia of other areas of the central nervous system. Studies of retinal progenitor cells have led to the identification of several factors that control their proliferation. It has been found that cell-type determination in the rodent retina is independent of lineage and that during the generation of retinal cell types, the cessation of mitosis and the cell-type determination are independent events, controlled by environmental interactions (Turner et al., 1990). Therefore, both intrinsic properties and extrinsic cues direct the choice of cell fate (Cepko et al., 1996). Progenitors pass through intrinsically determined competence states, during which they are capable of giving rise to a limited subset of cell types under the influence of extrinsic signals (Livesey and Cepko, 2001). On the other hand, the different retinal progenitor cells are heterogeneous with respect to their expression of cell-cycle regulators (Dyer and Cepko, 2001).

Each type of retinal cell is generated in a characteristic order with Müller glia, rod photoreceptors and bipolar interneurons being born last (Young, 1985). Some of the molecular signals that can influence the production of glia by retinal progenitor cells have been described, thus the basic helix-loop-helix gene, neuroD negatively regulates gliogenesis (Morrow et al., 1999). NeuroD promotes the development and/or survival of rods and amacrine cells, while suppressing two of the last born cell types, Müller glia and bipolar neurons. Recent work has focused on the role of p27kip1, a cyclin-dependent kinase inhibitor, in the genesis of Müller glia (Ohnuma et al., 1999) and it has been suggested that p27kip1 may collaborate with the notch pathway. Rax, Hes1 and notch1 are expressed in retinal progenitor cells and it has been found that Rax is expressed in differentiating Müller glia in the postnatal rodent retina. Moreover, the two other genes that are down-regulated in neurons, notch1 and Hes1, continue to be expressed in glial cells early in the development (Furukawa et al., 2000). Following neuronal damage, Müller cells undergo reactive gliosis characterized by proliferation and changes in gene expression. Downregulation of p27kip1 and re-entry into the cell cycle occurs within 24 hours after retinal injury whereas accumulation of p27kip1 correlates with cell-cycle withdrawal and differentiation (Levine et al., 2000).

In lower vertebrates, the retina continues to grow throughout the life of the animal. The new retinal cells are added at the ciliary margin of the eye from the mitotic activity of neural/glial stem cells in a region known as the germinal zone (Reh and Levine, 1998). Moreover, studies of retinal regeneration have shown that in cold-blooded vertebrates the neural retina is reconstituted by regenerative neurogenesis following its partial or total destruction. Two processes are involved in the retinal regeneration in fish and amphibians: transdifferentiation of retinal pigment epithelial cells into retinal neural progenitors and alteration in the fate of photoreceptor progenitors intrinsic to the retina (Hitchcock and Raymond, 1992). Thus, one source of regenerated retinal cells in teleost fish is a population of scattered proliferating cells located in the outer nuclear layer within the differentiated retina; these proliferating cells are modified neuroepithelial cells termed rod precursors because in the intact retina they produce only rod photoreceptor cells. When retinal neurons are destroyed, rod precursors cease producing rods and give rise to clusters of primitive neuroepithelial cells which divide and reconstitute the retina following a pattern that mimics the process of normal development (Raymond et al., 1988; Raymond, 1991).

Neural stem cells have been identified in the central nervous system and retina of adult birds (Goldman and Nottebohm, 1983; Fischer and Reh, 2000; Reh and Fischer 2001) and mammals (Reynolds and Weiss, 1992; Tropepe et al., 2000). It has been reported that stem cells at the retinal margin continue to produce new neurons through postnatal development and into adulthood in chick retina (Fischer and Reh, 2000). However, there are few reports of neural regeneration following acute damage in the central nervous system of warm-blooded vertebrates (Magavi et al., 2000; Scharff et al., 2000).

After neurotoxin-induced damage, proliferation is not increased at the chicken retinal margin (Fischer and Reh, 2000), but the central retina undergoes a massive proliferative response. Proliferating cells are Müller glia that re-enter the cell cycle, de-differentiate, acquire progenitor-like phenotypes, and produce new neurons and glia (Fischer and Reh, 2001). These findings suggest that some proliferating Müller glial cells are capable of de-differentiating into retinal progenitors and subsequently forming new retinal neurons, whereas others differentiate into Müller cells. The notion that glia may give rise to neurons is not without precedent, it has been proposed that transformation of Müller cells into

neural progenitors in the injured retina might occur in the fish retina. After laser ablation of photoreceptors in adult goldfish retina, microglia, Muller cells and retinal progenitors proliferate in the inner nuclear layer. The nuclei of Müller glia and associated retinal progenitors migrate from the inner to the outer nuclear layer. The proliferating Müller cells, which express Notch-3 and N-cadherin, do not generate extra glial cells in the region of the lesion so they must either die or transform into another cell type. It is possible that the progeny of the dividing Müller cells regenerate cone photoreceptors and then rod photoreceptors (Wu et al., 2001).

In other regions of the nervous system, evidence exists that glial cells can give rise to neurons. Thus, it has been found that neural stem cells in the adult mammalian brain are actually a subclass of glial cell, either specialized astrocytes in the subventricular zone (Doetsch et al., 1999) or ependymal cells at the ventricular surface (Johansson et al., 1999). Moreover, radial glial cells in the developing cerebral cortex in mammals can also behave as neural progenitors, even as they continue to express glial-specific markers such as GFAP (Misson et al., 1988).

Retina of postnatal chickens has the potential to generate new neurons, thus in response to damage, Müller glia lose their phenotype and de-differentiate into retinal progenitors. Proliferating Müller cells may express several genes expressed by progenitors, including CASH-1, Pax6 and Chx10 (Fischer and Reh, 2001). Newly-formed cells are distributed throughout the inner and outer nuclear layers of the retina. Some of them differentiate into retinal neurons, few form Müller glia, and most of them remain undifferentiated. These cells that do not differentiate, co-express pax6 and Chx10, and are in an arrested state. It is possible that the cues that induce embryonic progenitors to divide and differentiate are absent in mature retina, and, consequently, most of these cells remain in an arrested state (Fischer and Reh, 2001). Amacrine and bipolar cells can be generated from damage-induced progenitors; however no other retinal cell types are generated. In this sense, progenitors at the retinal margin of postnatal chickens primarily produce amacrine and bipolar cells (Fischer and Reh, 2000). This finding is consistent with the hypothesis that the microenvironment required to generate all cell types may be absent in the postnatal retina (Fischer and Reh, 2001). Recently, it has been demonstrated that a combination of insulin and FGF2 stimulates Müller glia to dedifferentiate, proliferate and generate new neurons (Fischer et al., 2002), suggesting that exogenous growth factors might be used to stimulate endogenous glial cells to regenerate neurons in the central nervous system.

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