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ROLE OF MÜLLER CELLS IN RETINAL DEGENERATIONS

Andreas Bringmann and Andreas Reichenbach

Paul Flechsig Institute for Brain Research, Leipzig University, Jahnallee 59, D-04109 Leipzig, Germany

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1. ABSTRACT

Müller (radial glial) cells span the entire thickness of the retina, and contact and ensheath every type of neuronal cell body and process. This morphological relationship is reflected by a multitude of functional interactions between retinal neurons and Müller cells, including extracellular ion homeostasis and glutamate recycling by Müller cells. Virtually every disease of the retina is associated with a reactive Müller cell gliosis. Müller cell gliosis may either support the survival of retinal neurons or accelerate the progress of neuronal degeneration. Müller cells are key mediators of nerve cell protection, especially via release of basic fibroblast growth factor, via uptake and degradation of the excitotoxin glutamate, and via secretion of the antioxidant glutathione. Neovascularization during hypoxic conditions is mediated by Müller cells via release of vascular endothelial growth factor and transforming growth factor β or via direct contact to endothelial cells. Primary Müller cell insufficiency has been suggested to be the cause of different cases of retinal degeneration including hepatic and methanol-induced retinopathy and glaucoma. It is conceivable that, in the future, new therapeutic strategies may utilize Müller cells for, e.g., somatic gene therapy or transdifferentiation of retinal neurons from dedifferentiated Müller cells.

2. INTRODUCTION: FEATURES AND FUNCTIONS OF MÜLLER CELLS

Müller cells are the principal glial cells of the neural retina. While retinal astrocytes surround blood vessels in the nerve fibre layer, Müller cells are the only macroglial cell which span the entire thickness of the retina and contact virtually all retinal neurons. Müller cells play a wealth of crucial roles in supporting neuronal survival and information processing (1,2). Müller cells are responsible for the structural stabilization of the retina. The layered arrangement of the retinal neurons is created and maintained by the Müller cell framework (3). Müller cells regulate the extracellular homeostasis of relevant ions including the pH, and of the water content of the extracellular space. The plasma membranes of Müller cells are highly permeable to K^+ (4,5) as a consequence of the strong expression of K^+ channels, especially of inwardly rectifying K^+ (Kir) channels (6-9). Active neurons release K^+ ions, and Müller cells siphon K^+ from the extracellular space and redistribute it into different sinks such as the vitreous body, blood vessels, and the subretinal space (10-12). Since the vitreous is a main sink for the K^+ redistribution, exchanging the vitreous body by media without the capability to dissolve K^+ ions (e.g. by perfluorocarbon liquid or silicone oil) should disturb the K^+ buffering function of Müller cells and should result in a neuronal degeneration (13). A prerequisite for effective K^+ distribution by Müller cells is a very negative membrane potential (normally around –80 mV) (4,14,15) which is maintained by open Kir channels (16).

Müller cells provide trophic substances to neurons (17) and remove metabolic waste. Müller cells metabolize glucose to lactate which is preferentially taken up by photoreceptors as a fuel for their oxidative metabolism (18). Therefore, the survival of retinal neurons depends on Müller cells. Selective Müller cell death causes retinal dysplasia, photoreceptor apoptosis and, finally, retinal degeneration and proliferation of the retinal pigment epithelium (RPE) (19).

Müller cells contribute directly and indirectly to the neuronal information process within the retina, particularly by fast uptake of released neurotransmitters and by providing precursors of neurotransmitters to the neurons. Müller cells play a crucial role in the glutamate-glutamine cycle within the retina. Müller cells (in addition

to retinal astrocytes and neurons) take up neuronally released glutamate. Müller cells dominate the total retinal glutamate transport (20). The primary glutamate transporter expressed by retinal astrocytes and Müller cells is the glutamate-aspartate transporter (GLAST) (21,22). The glutamate uptake by Müller cells is crucial for the rapid termination of the light-evoked activity in retinal ganglion cells (23), and also maintains the extracellular glutamate level at low values in order to prevent glutamate excitoxicity and disturbations of neuronal information processing. The Na^+-dependent glutamate uptake of Müller cells is electrogenic (24), i.e. a very negative membrane potential is necessary for efficient uptake (25). The expression and activity of GLAST in Müller cells is regulated by intracellular signaling pathways. Activation of the protein kinase C, e.g., after intracellular Ca^{2+} increase due to glutamate receptor activation (26), increases the uptake activity by both phosphorylation and increased expression of transporter proteins (27). This mechanism may be the cause of enhanced expression of GLAST by Müller cells under pathological conditions (28) when the intraretinal glutamate level is increased. Glutamate is degraded in Müller cells. There exist multiple glutamate-degradation pathways. The main pathways are the synthesis of glutamine by the glutamine synthetase and the synthesis of glutathione, which acts as an intraretinal antioxidant. Glutamine synthetase is a Müller cell-specific enzyme (29). It metabolizes the neurotoxic substances ammonia and glutamate (17). Demonstrable levels of glutamate are only present in Müller cells when the glutamine synthetase is experimentally inhibited (30) or down-regulated under pathological conditions (31,32). Glutamine is then transfered from Müller cells to neurons (33) and serves as a precursor for glutamate synthesis in the neurons, thus completing the glutamate-glutamine cycle. When the glutamine synthetase is experimentally blocked in Müller cells, retinal neurons lose their glutamate content, and the animals become rapidly (within 2 minutes) functionally blind (30,34). There may be also a Ca^{2+}-dependent glutamate release from Müller cells after stimulation which may directly modulate the activity of retinal neurons (35).

Under pathological conditions, Müller cells express the inducible NO synthetase (36). The NO synthesis by Müller cells may be involved in infectious (37) and ischemic processes, and in the pathogenesis of diabetic retinopathy (38). During hypoxia and hypoglycemia, NO may increase the retinal blood flow (38). The NO produced by activated Müller cells is not toxic for Müller cells themselves but may induce neuronal cell death (39,40).

Müller cells act as intraretinal modulators of immune and inflammatory responses. Müller cells inhibit the proliferation of T lymphocytes via a membrane-bound factor (41). Glucocorticoids can reverse the inhibition by suppressing the expression of the Müller cell inhibitory factor (42). Cultured Müller cells express MHC class II determinants in response to activated lymphocytes (43). In response to virus infection, Müller cells produce several proinflammatory cytokines (44).

In response to various different pathological stimuli, Müller cells show a reactive gliosis. Virtually every pathological alteration of retinal neurons affects the Müller cells and vice versa. However, whereas neurons are highly suseptible to injury, glial cells are more resistant, particularly to ischemia, anoxia, or hypoglycemia (45). In the presence of glucose and oxygen, cultured human Müller cells obtain their ATP principally (to 99 %) from glycolysis and display a low rate of oxygen consumption (18,46). This metabolic compartmentalization may save oxygen for retinal neurons, and also makes Müller cells resistant to anoxia. Moreover, short periods of glucose deficiency might be compensated by the glycogen deposits in Müller cells (47,48). On the other hand, cultured Müller cells are resistant to the absence of glucose since other substrates such as lactate, pyruvate, glutamate, or glutamine may be used as energy substrates for their - though normally non-dominant - Krebs cycle (46).

Müller cell gliosis is characterized by both unspecific and specific responses to pathogenic stimuli. The type of specific response is dependent on the type of the pathogenic stimulus while unspecific responses are shown by Müller cells under virtually all pathological conditions. There are three important unspecific responses: Müller cell hypertrophy and proliferation, and up-regulation of the immunoreactivity of the intermediate filaments vimentin, and, particularly, glial fibrillary acidic protein (GFAP). Regularly, in the healthy retina, vimentin is present in retinal astrocytes and Müller cells, while only astrocytes contain GFAP (49-54). Reactive Müller cells show an up-regulation of both types of intermediate filaments. The presence of an intraretinal pathological process is relatively easily and reliably detectable by the induction of GFAP expression in Müller cells, as the expression of GFAP by Müller cells is an early unspecific but highly sensitive indicator of a wide variety of retinal diseases and injuries (49). An up-regulation of GFAP was described, for instance, for mechanical injury (55), in light-induced photoreceptor degeneration (56-59), photoreceptor degeneration in aging Fischer rats (60); inherited retinal dystrophy of the Royal College of Surgeons (RCS) rats (61,62), glaucoma (63), retinal detachment (31,52,64-67), hepatic retinopathy (68,69), Alzheimer's disease (70), transient ischemia (53,54,71), and after lensectomy-vitrectomy (72). Similarly, Müller cell hypertrophy was described in different diseases including retinal light damage (59), retinal detachment (64,73), proliferative vitreoretinopathy (PVR) (15,74-76), and transient ischemia (77).

Specific Müller cell responses are dependent on the type of the pathogenic stimulus. A reduced expression of the glutamine synthetase is observed in Müller cells when the major glutamate-releasing neurons are lost, e.g., after retinal light damage (58) or after retinal detachment (65) which both cause photoreceptor degeneration. On the other hand, enhanced expression of the glutamine synthetase by reactive Müller cells is evident when its activity is necessary to detoxify the retina from elevated levels of ammonia (68).

Müller cell gliosis is a component of a complex retinal response to pathogenic stimuli which also includes

microglial activation, breakdown of the blood-retinal barrier (BRB) and an immigration of macrophages and lymphocytes into the retinal tissue, and which even may involve proliferation of cells of the vasculature. The processes of Müller cells (together with those of astrocytes which are restricted to the inner layers) surround all blood vessels within vascularized retinae. Müller cells produce factors capable of modulating blood flow and vascular permeability. They regulate the barrier properties of the BRB (78) which is mainly formed by the tight junctions between vascular endothelial cells (79). Glial cell line-derived neurotrophic factor (GDNF) and neurturin, which are secreted from retinal astrocytes and Müller cells, decrease the permeability of the BRB (80) while, during reactive gliosis, Müller cells may cause a leakage of the BRB. After breakdown of the BRB, the extravasated plasma protein IgG may further trigger the reactive gliosis of Müller cells (81), and serum components leaking into the perivascular environment may stimulate Müller cell proliferation (82). Müller cells participate in the unspecific defence and modulate specific immune responses. Müller cells are capable of phagocytosing fragments of retinal cells as well as foreign substances (83-86).

In dependence on the type of injury, Müller cells may aggravate or inhibit neuronal degeneration. Massive gliosis, accompanied by a long-lasting, uncontrolled Müller cell proliferation, is detrimential to the retina, while a ”conservative” reactive gliosis (87) accompanied by only a transient Müller cell proliferation may be helpful, e.g., by increased secretion of basic fibroblast growth factor (bFGF) or by a transdifferentiation into retinal neurons (88). In various diseases, Müller cells produce nitric oxide (NO). NO may be beneficial during bacterial infection and during ischemia where it increases blood flow. Moreover, low NO concentrations may protect neurons from glutamate excitotoxicity via closing of NMDA receptor-gated ion channels (89). By contrast, high NO concentrations have been implicated in neuronal cytotoxicity (39,40). Likewise, the effects of cytokines - often involving Müller cells - seem to depend on the specific type of retinal disease. For example, nerve growth factor (NGF) administration has been reported either to be beneficial (90) or to be detrimental (91) in different forms of retinal degeneration. After injury of the retina, Müller cells re-enter the proliferation cycle to establish a glial scar (92). Glial scars are one reason for the failure of the CNS to regenerate. During retinal detachment, for example, Müller cell processes grow through the outer limiting membrane and fill the spaces left by dying photoreceptors (73). Within the subretinal space, the Müller cell processes form a fibrotic layer that completely inhibits the regeneration of outer photoreceptor segments (73,93).

As examplified above, reactive gliosis is defined as a variety of Müller cell responses to events damaging primarily the retinal neurons. On the other hand, there are cases of retinal dystrophy in which Müller cells are the primary targets of the pathogenic stimuli. Müller cell insufficiency must cause impaired neuronal information processing due to failure of glial-neuronal interactions; these alterations finally cause visual deficits. Müller cell dysfunctions have been suggested as causes for visual impairments in hepatic retinopathy (68,69), in methanol-induced retinopathy (94), in a human disease caused by autoantibodies directed to Müller cells (95), in Müller cell sheen dystrophy (96), and in glaucoma (97,98). Müller cells were also implicated to be primarily affected in retinoschisis (99) and macular hole formation (100).

2. MÜLLER CELL-MEDIATED SUPPORT OF NEURONAL SURVIVAL

In the course of several retinal diseases and injuries including retinal detachment, hypoxia and hypoglycaemia of the outer retina occur and cause failure of ATP production in photoreceptors. The decreased energy supply causes programmed photoreceptor cell death (apoptosis) and a programmed destruction of the surviving photoreceptors (101). The photoreceptor death may be desaggravated by an injury-induced dispersal of bFGF from its normal storage sites in astrocytes, Müller cells, and neurons (101) because bFGF has been shown to exert a direct protective effect on photoreceptors (102-104). Other neurotrophic factors such as brain-derived neurotrophic factor (BDNF), ciliary neurotrophic factor (CNTF), and GDNF, have also been reported as protective for photoreceptor cells (67,103,105-107). The neurotrophic factor-mediated rescue of photoreceptors is likely mediated by an indirect mechanism via Müller cells (91) since a loss of Müller cells induces photoreceptor death (19), and Müller cells but not photoreceptors are activated by intraocular administration of BDNF, CNTF or bFGF (which is reflected in an increased phosphorylation of the extracellular signal-regulated kinase and in an increased c-Fos and GFAP staining) (108).

Generally, it was suggested that neurotrophins may control neuronal survival by two types of receptors: the Trk family of high-affinity tyrosine kinase receptors (TrkA, B, and C) transmit a prosurvival signal, while the low-affinity p75 neurotrophin receptor transmits an anti-survival signal (109). Müller cells are implicated in either photoreceptor cell death or rescue, in dependence on an increase or a decrease, respectively, of the release of the photoreceptor survival factor, bFGF. Experimental light damage of photoreceptors in the rat (110) is associated with increases in Müller cell expression of the p75 receptor and of the TrkC receptor, and with an induction of TrkC expression by photoreceptor cells (91). Neurotrophin-3 mediates its protective effect on photoreceptors by binding on the TrkC receptors of photoreceptors and of Müller cells, which latter event results in an increased release of bFGF from Müller glia (91). On the other hand, NGF reduces the bFGF production in Müller cells via binding on the p75 receptors; the reduced bFGF release by Müller cells may contribute to increased photoreceptor apoptosis (91). An open problem remains the precise mechanism how elevated intraretinal bFGF increase photoreceptor survival since the photoreceptors are not directly activated by this neurotrophic factor (108).

Additional (previous) retinal injuries like mechanical stress (102,111,112) or preconditioning with

bright light (113) may protect photoreceptors from degeneration because these stimuli cause an up-regulation of bFGF and CNTF expression by Müller cells (55,113). Likewise, argon laser photocoagulation slows photoreceptor degeneration in the RCS rat by induction of bFGF in retinal blood vessels, Müller cells and astrocytes (114). Similarly, the α2-adrenergic agonist-induced photoreceptor protection is mediated by an increased expression of bFGF by Müller cells (115).

In addition to that of photoreceptors, Müller cells may also provide a protection of other retinal neurons. BDNF indirectly increases the survival of retinal bipolar cells by acting through the p75 receptors of Müller cells which induces release of bFGF (116). NGF depresses the BDNF-promoted bipolar cell survival (116). The loss of ganglion cells after optic nerve transection in the frog retina is reduced by bFGF administration (117). In the rat retina after optic nerve transection, Müller cells express CNTF which is suggested to play a protective role for axotomized ganglion cells (118). Neuronal death induced by NMDA or kainic acid injections into the vitreous of rat eyes or transient ischemia induces an up-regulation of CNTF and GFAP expression in Müller cells (119,120). Pretreatment with CNTF has neuroprotective effects against glutmate excitotoxicity (120).

Retinae of aging Fischer rats display a gradual loss of photoreceptors which is accompanied by an increase of GFAP and carbonic anhydrase II content in Müller cells, and by an extension of Müller cell processes into the subretinal space (60). It was suggested that Müller cell gliosis may be the primary cause of the age-related retinal degeneration in Fischer rats since the increase of the Müller cell's GFAP content precede the photoreceptor degeneration in time and location (121).

3. INVOLVEMENT OF MÜLLER CELLS IN DISTINCT RETINAL DISEASES

3.1. Retinal detachment and proliferative retinopathies

Postmitotic Müller cells retain a remarkable plasticity even in adult retinae; they are capable of dedifferentiation and proliferation. In experimental retinal detachment, Müller cells become immediately reactive, with protein phosphorylation and increased production of transcription factors occurring within minutes after detachment (122). Within 3 hours after detachment, neuronal cell bodies are depleted of glutamate while Müller cells show an increased content of glutamine (123). Müller cell proliferation and increased GFAP and vimentin immunoreactivity begin within a day after detachment (124). After 3 days, Müller cell hypertrophy is obvious in the retina and in the subretinal space, and the Müller cell proliferation reaches its maximum (66,124). When no reattachment occurs, a retinal detachment may induce PVR and subretinal fibrosis (125). During retinal detachment, the distance between the vasculature in the choroidea and the photoreceptors increases. The detachment-induced Müller cell alterations have been suggested to be caused by hypoxia and hypoglycemia that result from impaired diffusion toward the outer layers of the detached retina from the choriocapillaris (126), likely secondary to photoreceptor degeneration. Transient ischemia also induces Müller cell poliferation (127). Müller cell proliferation and hypertrophy as well as photorecpetor degeneration are reduced by oxygen supplementation during experimental detachment (32,101). A reduction of glutamine synthetase activity in Müller cells of detached retinae (31,32) may underlie the accumulation of glutamate in these cells (32,128) which reflects a disruption of glutamate recycling and may contribute to glutamate excitotoxocity. The outgrowth of Müller cell processes into the subretinal space is preferentially associated with cone photoreceptors (73) which may indicate a specific interaction between the two cell types originating in early evolution (cones are phylogenetically older than rods [129], and the density of Müller cells is independent from rod density but closely related to cone density [130]). Müller cell hypertrophy and proliferation during detachment may be induced by bFGF released by cones (131) or by direct cone-Müller cell contacts (73).

In PVR and subretinal fibrosis, RPE cells, Müller cells, and astrocytes proliferate continuously for weeks or months (124,132) and migrate onto the retinal surfaces. During PVR, cells attach to the internal limiting membrane, proliferate, and form epiretinal "cellular membranes". Subsequent contraction of the cellular membranes produces recurrent retinal detachment. Since Müller cells in culture have the ability to contract (133,134) it is likely that Müller cells within the epiretinal membranes (135,136) participate in the contraction of these membranes.

In response to experimental retinal injury, Müller cells down-regulate their expression of the tumor suppressor protein $p27^{Kip1}$ which results in Müller cell proliferation (137). Müller cell proliferation is transient and reaches a maximum at 1 day after injury. Then, proliferation ceases due to down-regulation of cyclin D3 which is accompanied by an up-regulation of GFAP. The down-regulation of $p27^{Kip1}$ may represent one of the first molecular markers for the induction of reactive gliosis, even before GFAP up-regulation. Cyclin D3 down-regulation is assumed to prevent uncontrolled Müller cell proliferation (137). Transient Müller cell proliferation induced by NMDA excitotoxicity in postnatal chicken retinae causes a dedifferentiation of Müller cells into retinal progenitor-like cells (88). This progenitor-like cells may remain undifferentiated for longer periods and may then generate new Müller cells, or may even transdifferentiate into retinal neurons (amacrine and bipolar cells). Therefore, Müller cells are a potential source of neural regeneration within the postnatal retina (88).

A dedifferentiation of Müller cells during retinal detachment and PVR is also evident in electrophysiological studies (138). Müller cells from detached rabbit retinae show a decrease in their Kir currents (75) while Müller cells from PVR retinae display virtually no Kir currents; this is accompanied by a depolarization of the membrane potential (15,74,75). On the other hand, the amplitude of fast Na^+ currents (139) and the activity of Ca^{2+}-dependent K^+ channels, which are necessary for proliferation of

Müller cells (82,140,141), are enhanced (15). Both the decrease of the Kir currents and the increased activity of Ca^{2+}-dependent K^+ channels reflect a dedifferentiation of Müller cells (87) while the Na^+ current increase might indicate a transdifferentiation towards neuron-like cells. A down-regulation or closure of Kir channels in Müller cells must have deleterious consequences for retinal functions since Kir channels are assumed to mediate spatial buffering of the extracellular K^+ concentration (2,10). Furthermore, the activity of Kir channels causes the very negative membrane potential of Müller cells (16) essential for the activity of electrogenic neurotransmitter uptake carriers, e.g., for glutamate (142,143) and for GABA (144,145). Therefore, the down-regulation of Kir channels in Müller cells during PVR should cause an over-excitation of the surrounding neurons due to elevated extracellular K^+ and glutamate levels.

3.2. Retinitis pigmentosa

The loss of photoreceptors in retinitis pigmentosa is usually followed by alterations in the RPE and in the retinal glia. However, there are also indications that Müller cells may be causally related to the photoreceptor death. In an autosomal recessive retinitis pigmentosa, a mutation of the gene encoding cellular retinaldehyde-binding protein (CRALBP) was described (146). CRALBP is not expressed in photoreceptors but is abundant in the RPE and in Müller cells, where it carries 11-cis-retinol and 11-cis-retinaldehyde. The mutant protein lacks the ability to bind 11-cis-retinaldehyde. The lack of functional CRALBP may lead to a disruption of retinal vitamin-A metabolism (146). In RCS rats, the content of glutamine and arginine is elevated in Müller cells prior to the onset of photoreceptor death, due to anomalies in glutamate degradation (147).

3.3. Neovascularization

Neovascular diseases of the retina are a major cause of blindness. Several retinal and choroidal diseases such as diabetic retinopathy, retinopathy of prematurity, retinal vein occlusion, and the wet form (but not the dry form) of age-related macular degeneration are accompanied by pathological intraocular neovascularization. Hypoxia is thought to be a common precursor to neovascularization in many retinal diseases. Müller cells (in addition to astrocytes and RPE cells) may play a crucial role in the induction of hypoxia-induced neovascularization. This may be mediated by multiple mechanisms. The vascular endothelial growth factor (VEGF) is a key regulator of normal and pathological retinal angiogenesis (148-152), and is produced and released by Müller cells (150,153-157). Hypoxia is an important stimulus for both retinal VEGF synthesis and neovascularization (156,158); furthermore, intracellular ATP depletion (e.g., due to glucose deprivation) may stimulate VEGF synthesis (159). Müller cells are relatively resistent against hypoxia since their energy metabolism mainly relies upon anaerobic glycolysis (18,46,48) while the neuronal activity is oxygen-dependent. During hypoxia, the intraneuronal ATP may be not completely regenerated; therefore, adenosine (160), AMP or ADP may be released from retinal neurones. AMP and ADP can activate purinergic P2 receptors on Müller cells, an event which may result in an increased synthesis and release of vasoendothelial growth factor(s). Moreover, adenosine stimulates the intraretinal production of VEGF (161). The release of VEGF by cultured Müller cells is increased during hypoxia (153,156,157); the stimulatory effect of hypoxia is blocked by high concentrations (10-25 mM) of external glucose (157). Probably, the additional glucose supply fuels the glycolytic pathway and prevents the ATP depletion in Müller cells.

Müller cells from humans with diabetes or with disciform age-related macular degeneration show an increased expression of VEGF (162). VEGF expression in Müller cells precedes neovascularization in the retinae of humans with diabetes, at times when there is no anatomic evidence of retinal malperfusion (162). This indicates that ischemia may not be the only stimulus for VEGF expression in Müller cells (162). Moreover, it is likely that VEGF also fulfills functions other than stimulation of neovascularization. Müller cells express the VEGF receptors 1 and 2 (163,164), and activation of these receptors is necessary for regular retinal development, independent of vascularization (164).

It is likely that more than one growth factor contribute to hypoxia-induced pathologic angiogenesis. The glial, vascular endothelial and RPE cells present in human retinal neovascular membranes express both VEGF and bFGF (165). A second way for Müller cell-induced stimulation of neovascularization is the release of TGF-β and bFGF. Astrocytes and Müller cells express TGF-β (166). Cultured rat Müller cells synthetize and release TGF-β and VEGF (166,167). Under normoxic conditions, Müller cells release latent TGF-β, while in hypoxia, the active form of TGF-β is released (166). TGF-β stimulates the VEGF expression by Müller cells (166). TGF-β and/or a direct contact of astrocytes or Müller cells to retinal capillary endothelial cells induce the production and the release of the matrix metalloproteinase-9 (MMP-9) by the endothelial cells (168). The secretion of MMPs allows endothelial cells to penetrate their underlying basement membrane (169), generates leaky vessels, and eliminates the contact inhibition which normally blocks endothelial cell proliferation (170). In addition to TGF-β, the angiogenic factors VEGF and bFGF also up-regulate MMPs in cultured endothelial cells (171-173). The TGF-β-induced release of MMP-9 causes the BRB breakdown occurring at the onset of angiogenesis (168). The TGF-β induced and/or contact-mediated expression of MMPs in endothelial cells may be a way how astrocytes and Müller cells regulate the barrier properties of retinal blood vessels (78) via regulation of the expression of the tight junction protein, occludin, in endothelial cells (168).

A third way how Müller cells stimulate vasculogenesis and angiogenesis is present at least during ontogenetic development, and involves the activation of adenosine A2a receptors which are expressed by the developing blood vessels and by angioblasts (174). Müller cells stimulate vasculogenesis by producing adenosine via the ectoenzyme 5'-nucleotidase which is expressed on inner Müller cell processes during the period in which

angioblasts and endothelial cells express high levels of adenosine A2a receptors (175).

A further way for Müller cells to stimulate vasculogenesis may involve the renin-angiotensin system which has been implicated in diabetes. Müller cells in rat and human retinae express renin, with the expression being most obvious in endfeet closely apposed to retinal blood vessels (176).

3.4. Diabetic retinopathy

Chronic diabetes is associated with structural alterations in retinal capillaries. The general view is that acellular occluded vessels cause hypoxia which stimulates abnormal angiogenesis. Müller cells grow into the former lumen of occluded retinal vessels where they form a glial scar (177). In early diabetic rats, apoptosis occurs primarily in ganglion and Müller cells (90). This is associated with an up-regulation of Müller cell GFAP content and an increased expression of the p75 receptor on both cell types (90,178). NGF treatment of diabetic rats prevented apoptosis in ganglion and Müller cells as well as the development of pericyte loss and acellular occluded capillaries (90). In the rat retina, NGF is expressed by ganglion, Müller, and RPE cells while only RPE and Müller cells express NGF receptors (179). The glial cell derived factors VEGF ("vascular permeability factor") or TGF-β (168) may cause a leakage of the BRB and, therefore, edema in diabetic maculopathy. In diabetes, the lack of insulin should cause a depletion of glycogen stores in Müller cells (1). Furthermore, insulin seems to be necessary for the insertion of Kir channels into the Müller cell membrane (9) which play a crucial role in the maintenance of extracellular K^+ homeostasis and in supporting glutamate uptake by Müller cells. An involvement of insulin, which is intraretinally produced by Müller cells (180), in diabetic retinopathy remains to be established.

In experimental (streptozotocin-induced) diabetes in rats, glial reactivity is an early feature of diabetic retinopathy. The density of Müller cells is increased at 4 weeks of diabetes while the number of astrocytes is reduced (181). GFAP expression in Müller cells is prominent at 12 weeks (181,182). A leakage of the BRB is present already at 2 weeks of diabetes, i.e., before Müller cell reactivity is morphologically apparent (181). The BRB leakage is correlated with a reduction of the tight junction protein, occludin, in endothelial cells (183). At 12 weeks, the conversion of glutamate into glutamine is reduced within the diabetic retina, resulting in an increased retinal glutamate content (182). Because Müller cell reactivity and decreased glutamate metabolism of Müller cells are relatively early pathologic events, the involvement of early glutamate excitotoxicity in the course of diabetes has been suggested (182). In contrast to experimental diabetes in rats, no glutamine synthetase alterations were described in human diabetic retinopathy (178). In retinae of streptozotocin-induced diabetic rats, VEGF mRNA is present in various cell types including vascular and Müller cells (154).

In non-diabetic rat and human retinae, advanced glycation end products (AGEs) are only present at moderate levels in glial and vascular structures while in diabetic retinae, the expression of AGEs is strongly increased (184). The AGE receptor is expressed by Müller cells (184). AGEs induce the expression of VEGF by cultured Müller cells (155). The vitreous concentrations of AGEs and of VEGF are both elevated in patients with proliferative diabetic retinopathy. Elevation of AGEs in the vitreous may promote intraocular neovascularization in diabetic retinopathy via release of VEGF by Müller cells (155).

3.5. Glaucoma and transient ischemia

Low concentrations of excitotoxic agents, such as glutamate and NO, decrease the survival rate of ganglion cells (by increased apoptosis). Müller cells may protect ganglion cells from glutamate and NO toxicity, particularly via glutamate uptake and subsequent detoxification by glutamine synthetase (21,98,185-187). Glutamate exerts a direct excitotoxic action as well as an indirect effect, via stimulation of the synthesis and release of NO. NO has been implicated in a number of retinal diseases, including glaucoma and ischemia (38,188). Glutamate is increasingly released from neurons during retinal ischemia (189) while the glutamate uptake by Müller cells is reduced due to a high extracellular K^+ concentration which leads to a saturation of the uptake already at low glutamate concentrations (190). Functional disorders of the glutamate uptake in Müller cells may be one of the etiologies of glaucoma, especially in patients with satisfactory control of intraocular pressure (97,98). It has been suggested that stimulation of Müller glial glutamate uptake might prevent excitotoxic damage and subsequent generation of other excitotoxins, such as NO, during glaucoma (98).

Potentially damaging reactive oxygen species are generated in the retina under various conditions, such as anoxia and ischemia (191); they can provoke cell necrosis or trigger apoptosis. Reactive oxygen species also inhibit the Na^+/K^+-ATPase, which may lead to ionic imbalance and to an inhibition of the glutamate transporter, for example (191). One of the crucial substances protecting the retina against reactive oxygen species is reduced glutathione, a tripeptide constituted of glutamate, cysteine, and glycine. Glutathione is exclusively synthetized in Müller cells (192,193), and glutamate is the rate-limiting substance (194). During hypoxia and hypoglycemia, the glutathione levels in acutely isolated Müller cells decrease dramatically (194); a fast transfer of glutathione from Müller cells to neurons, particularly to ganglion cells, has been suggested (193). Generally in Müller cells, glutamate is preferentially converted to glutamine rather than into glutathione (194). During glutamate deficiency, e.g. due to an impaired glutamate uptake during an ischemia-induced increase of the extracellular K^+ concentration, this may result in a glutathione deficiency. The ischemia-induced lack of glutathione may increase the intraretinal level of reactive oxygen species. Müller cells from aged guinea-pigs contain significantly less reduced glutathione than cells from young animals (195) indicating an involvement

of decreased glutathione also in age-related retinal degenerations.

3.6. Hepatic retinopathy

Patients with liver insufficiency display high levels of serum ammonia which are assumed to be causally related to the development of hepatic retinopathy (68,196). In this disease, the primary pathological alterations are found in the Müller cells and retinal astrocytes (68), similar to the occurrence of Alzheimer-type II astrocytes in the brain (197). Characteristic Müller cell alterations such as cell swelling, vacuolization, increased GFAP and glutamine synthetase content, loss of Kir currents and membrane depolarization are inducible *in vitro* by enhanced levels of ammonia (196,198,199). Similar alterations were observed *in vivo* in rats with liver insufficiency (69). By contrast, retinal neurons display no apparent morphological changes. The glutamine synthetase in Müller cells and astrocytes is the only enzyme capable of ammonia detoxification within the retina. The activity of the glutamine synthetase requires energy (17). A dramatically enhanced metabolic activity, caused by the necessity to detoxify ammonia and reflected by an enlargement of cell nuclei (68), may produce a decreased energy state in Müller cells. Indeed, glycogen depletion (200) and swollen mitochondria (69) have been observed. Müller cell damage in hepatic retinopathy may underlie the occurrence of retinal symptoms which have been electroretinographically revealed on patients with liver insufficiency (201). The necessity to detoxify the retina from ammonia results in an enhanced glutamate consumption for glutamine synthesis; the competition with glutathione formation may cause a lack of glutathione, and thus may accelerate pathogenic mechanisms involving free radicals (48). A similar pathogenetic mechanism (i.e., based on energy depletion in Müller cells caused by the need to detoxify the retina) has been suggested for the methanol-induced retinopathy (94).

3.7. Retinoschisis

In retinoschisis, cystic degeneration (mainly in the deep nerve fiber layer) causes intraretinal splitting. Though the mutant gene in human X-linked retinoschisis is probably not directly related to Müller cells, an involvement of Müller cells in the pathogenic mechanism(s) of the disease has been proposed (202,203). First, deposits of amorphous filamentous material have been found within degenerated Müller cells as well as in adjacent extracellular spaces (204). It has been suggested that these extracellular filaments are of glial origin; the idea is that defective Müller cells produce GFAP and S-100 protein which accumulate within the retina, and secondarily result in degeneration of the cells and thus - due to loss of the mechanical scaffold - in the occurrence of schisis (204). Second, though the mutation responsible for human X-linked retinoschisis has been localized in the gene coding for the protein, retinoschisin (RS1) which is expressed in photoreceptor and bipolar cells rather than in Müller cells (205), it has recently been shown that retinoschsin is selectively taken-up and transported by Müller cells into the inner retina in a direction-specific manner (206). Apart from any direct involvement of Müller cells in the pathomechansms of retinoschisis, impaired Müller cell functions must result from the massive elongation of the cells. An excess of extracellular K^+, due to a decreased K^+ siphoning capacity of Müller cells, has been suggested to cause the Mizuo-Nakamura phenomenon in retinoschisis (99). Insufficient clearance of K^+ ions (and, in turn, depolarization-mediated inhibition of glutamate uptake) should greatly aggravate neurodegeneration within the cystic tissue.

4. PERSPECTIVES

There is now increasing knowledge about the Müller cell involvement in virtually all types of retinal degeneration; this may support the development of new therapeutic strategies to prevent retinal dystrophy or to substitute degenerated retinae by retinal cells generated in culture. Because Müller cells show a high resistance against various pathogenic stimuli, they may play a central role in such novel therapeutic approaches. In the future, several different therapeutic strategies are conceivable, all based on Müller cells. For example, somatic gene therapy may be carried out via Müller cells (207). When particular types of neurons (e.g., photoreceptors or ganglion cells) degenerate, a gene transfer to Müller cells may help to support their protective role on photoreceptor survival. Müller cells were found to be primarily transfected when genes, e.g. the BDNF gene, were delivered by adenoviral vector-injections into the vitreous chamber (207-209). Because Müller cells span the entire thickness of the retina, adenovirus-mediated gene delivery to these cells should be useful to modulate the survival of all neuronal cell types within the retina (209). It is also conceivable that a therapeutic induction of intraretinal Müller cell proliferation may be helpful to replace degenerating neurons by transdifferentiated Müller cells (88); application of transgenes may guide the direction of the transdifferentiation, and may even determine specific neuron types developing from proliferating Müller cells. Degenerated parts of the retina or even the whole retina may be replaced by retinae grown from multipotential stem cells *in vivo* or *in vitro*. There are possibilities for the re-initiation of retinal stem cell potential at the ciliary margin of the eye (210), from the RPE (211-213), or from Müller cells (88,214). Perhaps, glial scars, e.g. epiretinal membranes in PVR eyes, may be a source to obtain de-differentiated Müller cells from which new retinae would then be formed *in vitro*. However, these are pies in the sky, requiring that more informations will be obtained about Müller cell functions in the normal and in the diseased retina.

6. ACKNOWLEDGEMENTS

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Abbreviations: AGE, advanced glycation end product; ADP, adenosine 5'-diphosphate; AMP, adenosine 5'-monophosphate; ATP, adenosine 5'-triphosphate; BDNF, brain-derived neurotrophic factor; bFGF, basic fibroblast growth factor; CNTF, ciliary neurotrophic factor; CRALBP, cellular retinaldehyde-binding protein; BRB, blood-retinal barrier; GABA, γ-aminobutyric acid; GDNF, glial cell line-derived neurotrophic factor; GFAP, glial fibrillary acidic protein; GLAST, glutamate-aspartate transporter; Kir, inwardly rectifying K^+; MMP, matrix metalloproteinase; NGF, nerve growth factor; NMDA, N-methyl-D-aspartate; NO, nitric oxide; PVR, proliferative vitreoretinopathy; RCS, Royal College of Surgeons; RPE, retinal pigment epithelium; TGF-β, transforming growth factor β; VEGF, vascular endothelial growth factor

Key Words: Müller Cell, Glia, Reactive Gliosis, Ionic Homeostasis, Transmitter Recycling, Trophic Factors, Proliferative Retinopathies, Neovascularization, Retina Degeneration, Ischemia, Hepatic Retinopathy, Retinoschisis, Review

Send correspondence to: Prof. Dr. Andreas Reichenbach, Paul Flechsig Institute of Brain Research, University of Leipzig, Jahnallee 59, D - 04109 Leipzig, Germany, Tel.: +49-341-9725 731, Fax: +49-341-9725 739, E-mail: reia@medizin.uni-leipzig.de