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Müller glial cells – the mediators of vascular disorders with vitreomacular interface pathology in diabetic maculopathy

Komórki glejowe Müllera – mediatorzy zaburzeń naczyniowych z patologią złącza szklistkowo-plamkowego w makulopatii cukrzycowej

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Summary: The key to identifying the type of diabetic maculopathy is determining the status of posterior vitreous adhesion. In the pathological state, the breakdown of the internal and external blood-retina barrier is evident, however the mechanism is usually complex. The common denominator for these disorders are Müller glial cells, which mediate in maintaining the blood-retina barrier by linking the vessels, neurons and the vitreous in anatomical network and into functional dependence. The breakdown of the blood-retina barrier results in proliferation of Müller cells. Molecular changes in these cells increase endothelial barrier properties, but also induce pathological processes on the vitreo-retinal junction, resulting in increased adhesiveness of the collagen fibers of vitreous to retinal internal limiting membrane. The ability of Müller cells to reactive gliosis is influenced by the healthy functioning of the retinal pigment epithelium, which is a source of trophic factors necessary for appropriate Müller cells morphogenesis. Vitrectomy with the removal of ILM eliminates the vitreofoveal interface pathology, additionaly provoking reactive gliosis within the macula. Intraoperative use of anti-VEGF supports short-term tightness of the blood-retina barrier in the perioperative neuralgic period. In the future, supplying astrocytes may be a strategy that will allow not only the inhibition of pathological neovascularization but also the restoration of the physiological network of capillaries in avascular retina areas. The delivery of recombinant PEDF allows for the recovery of Müller cells, and thus creates the conditions favourable for the survival of nerve cells in loss of retinal homeostasis.

Słowa kluczowe: makulopatia cukrzycowa, komórki glejowe Müllera, bariera krew–siatkówka, połączenia ciasne tight-junctions.
Key words: diabetic maculopathy, Müller glial cells, blood–retinal barrier, tight-junctions.

Blood-retina barrier

The blood-retina barrier is essential in preserving retinal homeostasis (1). It controls the flux of fluid, ions, and metabolites of retinal vascular origin to the retina, helping to stabilize the neuronal environment, necessary for the proper function of the nervous tissue. This is achieved internally, by close association of glial cells with endothelial cells in the capillaries and arterioles traversing the retina, together with the monolayer of retinal pigment epithelium cells, externally. This barrier is composed of a junctional complex, that includes tight and adherens junctions. The unique barrier properties of the retinal vessels are the result of close association between the endothelia and astrocytes in the inner retina and Müller cells in the capillary plexuses of the outer retinal layers. The restrictive control of blood elements in the retinal matrix is essential due to a number of reasons.

The most important are:

1. Constant exchange of metabolites between glia and neurons.
2. Strict control of the ionic environment, which allows neurons to establish and control membrane potentials and for the appropriate conduction of nerve impulses.
3. The blood contains amino acids and metabolites, which are employed by neural tissue as signalling molecules, for example aspartate and glutamate. Their concentration in blood is relatively higher than in the synaptic space.

Strict maintenance of the blood-retina barrier, as mentioned before, depends on close connection of glial cells with endothelial cells and arterioles penetrating the neural tissue on one side and neurons on the other. Many experimental studies give evidence for glial induction of endothelial barrier properties (2-7). The ability of glial cells to induce properties of endothelial barrier suggests that the breakdown of the blood-retina barrier in eye diseases may be a result of changes in glial function or loss of communication with endothelial cells. Diabetic maculopathy is characterized by accumulation of extracellular fluid initially in the Henle fiber layer, in the internal nuclear layer of the retina as well as in the subretinal space (Fig. 1).

The loss of the blood-retinal barrier includes increased permeability in both the blood vessels and the retinal pigment epithelium. Whereas altered vascular permeability appears to precede changes in the pigment epithelium in diabetes (8).

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Fig. 1. Diffuse diabetic macular edema with accumulation of fluid in the Henle fiber layer, in the internal nuclear layer of the retina, as well as in the subretinal space (own material).

Ryc. 1. Rozlany cukrzycowy obrzęk plamki z nagromadzonym płynem w warstwie włókien Henlego, w warstwie jądrzastej wewnętrznej siatkówki, jak również w przestrzeni podsiatkówkowej (materiał własny).

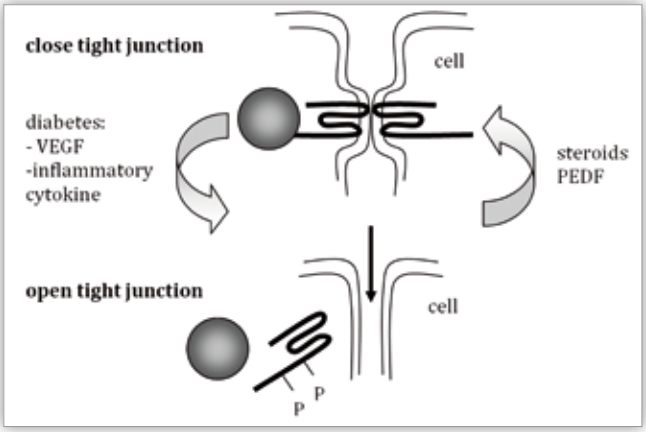

Fig. 2. The redistribution of tight junction proteins at the cell surface. In diabetes, VEGF as well as inflammatory cytokines cause phosphorylation of occludin and tight junctions disassembley. Steroids induce the synthesis of tight junctions proteins, dephosphorylation of occludin and assembley of tight junctions at the cell surface. Adapted from (1).

Ryc. 2. Redystrybucja białek połączeń ciasnych z powierzchni komórki. W przypadku istnienia cukrzycy produkcja zarówno VEGF, jak i cytokin zapalnych powoduje fosforylację okludyny i demontaż połączeń ciasnych. Steroidy indukują syntezę białek połączeń ciasnych, defosforylację okuldyny i montaż połączeń ciasnych na powierzchni komórki. Zaadaptowane z (1).

The mechanism of injury is based on the redistribution of the proteins forming tight junctions, mainly occludin (Fig. 2). The most important biological transmitter responsible for these changes is the vascular endothelial growth factor (VEGF). Its sources are the glial cells, activated monocytes, vascular endothelial cells and retinal pigment epithelium. The strongest inducer of VEGF in cells is hypoxia. VEGF expression in the retina occurs before the onset of proliferative retinopathy, suggesting a role for this growth factor specifically in vascular permeability (9).

Anatomy of the central retina

The particular role of glial cells in developing macular edema corresponds with the structural construction of the central retina (Fig. 3).

A particularly large number of Müller cells processes were found at the center of the fovea (10). These cells contain a watery, transparent cytoplasm and make up the scaffolding for the retinal neurons and the only central photoreceptors fibers junction in the macular area of about 50 μm. Each foveal cone is surrounded by Müller cell processes, that at the external limiting membrane form a 0,1 mm horizontal microvillis, which surround the base of each inner segment of the cone. This creates a unique, stable, in physiological conditions, glial-photoreceptor mozaic with constant retinal resolution. At the central region of the fovea (dimension about 200 μm), ILM is extremely thin and is lacking the inner nuclear layer, inner plexiform layer, ganglion cells and the nerve fiber layer. Therefore, this area is mostly composed of foveal cones and Müller cells. Müller cell's delicate, watery cytoplasm, in the electron microscope image, suggests that they are rich in polysaccharides or glycoproteins. Therefore, they not only facilitate the light to transpierce the retinal thickness, but also quickly collect liquid which leads to macular edema. The resorbtion of the edema fluid is further hampered due to the lack of capillaries in the avascular macular area. In the past, light microscopy showed a lack of Müller cells in the macula due to their destruction during the preparatory procedures (11). Lastly, the relatively high content of Müller cells in the central macular region is worthy of special attention.

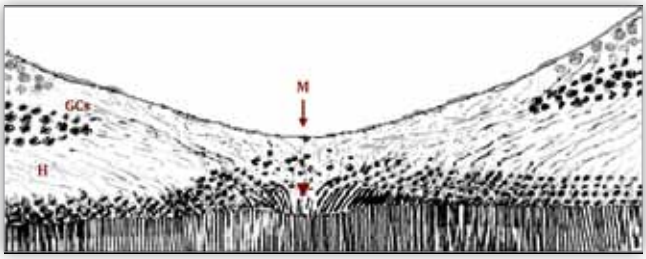

Fig. 3. Anatomy of the fovea centralis. Müller cell cone (M) whose base (arrow) corresponds with the internal limiting membrane, and whose apex corresponds with the outer membrane limiting centrally (arrowhead). Henle nerve fiber layer (H) and foveal edge of the ganglion cell layer (GCs) are shown. Adapted from (10).

Ryc. 3. Anatomia dołeczka centralnego. Wyrostki komórek Müllera, których podstawa koresponduje z błoną graniczną wewnętrzną (strzałka), a szczyt łączy się z błoną graniczną zewnętrzną (grot strzałki). Widoczna warstwa włókien nerwowych Henlego (H) i brzeg warstwy komórek zwojowych (GCs). Zaadaptowane z (10).

Choroidal circulation

The posterior ciliary artery (PCA) circulation is the main source of blood supply not only to the optic nerve head and the choroid up to the equator, but also to the retinal pigment epithelium (RPE) and the outer 130 μm of the retina (and in case, when a cilioretinal artery is present, the entire thickness of the retina in that region). That makes PCA circulation the most important part of ocular circulation. Therefore, disturbances in PCA circulation can result in a variety of ocular vascular disorders, causing varying degrees of visual loss. The macular region, in a particular way, is dependant on the choroid blood flow because it lies within the watershed zones between SPCAs, which correspond with choroid lobules (12).

The contraction of afferent arterioles in hypertension may result in central retinal hypoxia and, consequently, in the creation of macular edema, because choriocapilaries are in fact the only source of nutrition for the pigment epithelium and the retina in the macular area. Diffuse edema of the retinal area may correspond with the reduced choroidal lobules in the blood flow. The extent of damage to choriocapilaries perfusion depends on the caliber of contracted vessels (Fig. 4). Tissue hypoxia results in increased expression of vascular endothelial growth factor in retinal cells (pigment epithelium, pericytes, Müller cells and endothelial cells), which causes a disturbance of the blood-retina

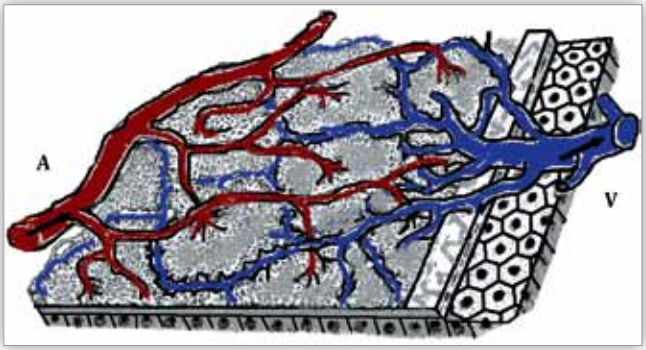

Fig. 4. Diagrammatic representation of choriocapillaris. A – choroidal arterioles, V – choroidal vein. Adapted from (12).

Ryc. 4. Schematyczne przedstawienie choriokapilarów. A – tętniczka naczyniówkowa, V – żyłka naczyniówkowa. Zaadaptowane z (12).

barrier and formation of macular edema in diabetic patients (13--15). Moreover, it was also demonstrated that the choroid blood flow is significantly lowered in patients with type 2 diabetes and diabetic macular edema (16).

Therefore, the macular status in diabetic retinopathy depends not only on damaged capillaries around the macula but also on the efficiency of choroid circulation in the submacular area. The pathological changes depend on the maintenance of the pigment epithelium and the production of PEDF (pigment epithelium-derived factor). It has been proven that this factor has neuroprotective properties. It prevents the degeneration of photoreceptor and maintains proper Müller cells ultrastructure, which produces adherent junctions between photoreceptors (17).

Retinal circulation

The human retina has a unique vascular structure, which requires migration of endothelial cells and pericytes in the retinal nerve tissue while preserving the blood-retina barrier. The entire retina is maintained in controlled isolation from the choroidal vascular compartment by tight-junctions in a single layer of retinal pigment epithelial cell board. Within the retina, this barrier is implemented mainly through adherent junctions of Müller cells processes ensheathing the vascular endothelium. The retinal capillaries are organized in three capillary levels (Fig. 5) (18). The first branches (c1) at the interface between the retinal ganglion cells and the inner plexiform layer, the second (c2), in the vicinity of the amacrine cells and the third (c3), exactly at the level of the horizontal cells in the outer plexiforme layer. It is important to note that the blood-retinal barrier in capillary circulation c1 and c2 is provided almost exclusively by Müller cells, whereas at c3 this barrier is carried equally by Müller cells and horizontal cells (19). The horizontal cells are considered to be interneuron inhibitors and they are located in the outer retina. They have the shape of glial cells. In the central, rodless region of the retina, the horizontal cells do not possess axons, and through the processes are in direct contact with capillary endothelial cells, which further strengthen their glial properties. Damage to these cells at an early stage of diabetic retinopathy may be related to the breakdown of the blood-retina barrier at the c3 capillary level and to the accumulation of fluid, mainly in the outer plexiform layer. A recently characterized form of neovascularization is evidently derived from

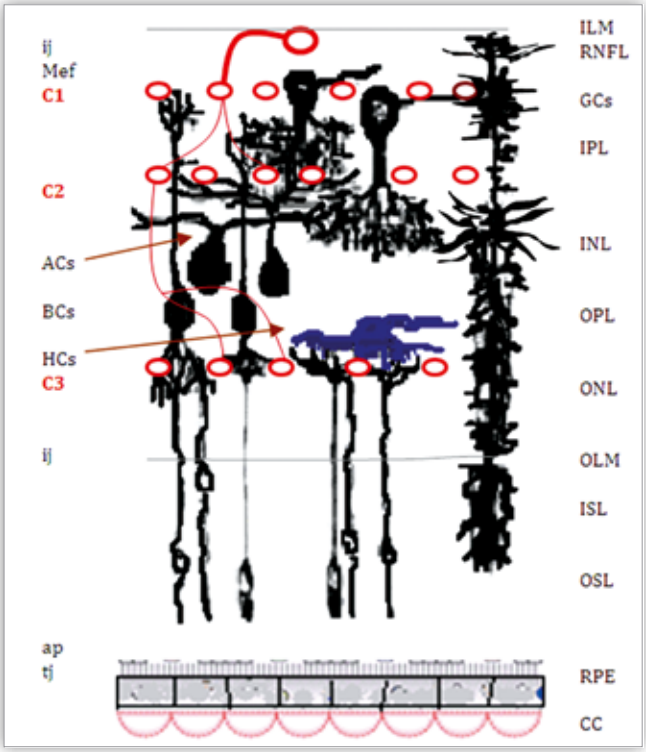

Fig. 5. Relations among retinal capillaries and retinal neurons and the principal glial cell – Müller cell. Adapted from (18).

Abbreviations: RPE – retinal pigmented epithelium, OSL/ ISL – outer/ inner segment layer, OLM/ ILM – outer/ inner limiting membrane, ONL/ INL – outer/ inner nuclear layer, OPL/ IPL – outer/ inner plexiform layer, tj/ ij – tight/ intermediate junctions, AC – amacrine cell, BC – bipolar cell, GC – ganglion cell, HC – horizontal cell, MC – Müller cell, c1/ c2/ c3 – capillary arcades of the GC/ AC/ HC layers, RNFL – retinal nerve fiber layer, ap – apical processes, mef – Müller cell end feet.

Ryc. 5. Relacje naczyń włosowatych siatkówki z neuronami siatkówki i główną komórką glejową – komórką Müllera. Zaadaptowane z (18).

Skróty: RPE – nabłonek barwnikowy siatkówki, OSL/ ISL – warstwa członów zewnętrznych/ wewnętrznych, OLM/ ILM – błona graniczna zewnętrzna/ wewnętrzna, ONL/ INL – warstwa jądrzasta zewnętrzna/ wewnętrzna, tj/ ij – połączenia ciasne/ pośrednie, AC – komórka amakrynowa, BC – komórka dwubiegunowa, GC – komórka zwojowa, HC – komórka pozioma, MC – komórka Müllera, c1/ c2/ c3 – arkady włosniczkowe na poziomie GC/ AC/ HC, RNFL – warstwa włókien nerwowych siatkówki, ap – wyrostki szczytowe, mef – końcowe wypustki komórek Müllera.

the c3 plexus and is called RAP – retinal angiomatous proliferation (20). It is possible that the development of RAP is linked with the loss of horizontal cells. The second neuroglial class besides Müller cells are astrocytes. They reside in the retinal nerve fiber layer, particularly around the optic nerve head and they sometimes also surround the capillaries in the c1 capillary plexus.

Retinal glial remodelling

It has been shown in animal models of diabetes that the breakdown of the blood-retina barrier induces reactive gliosis (21). Müller cells undergo hyperplasia preceding GFAP expression, and microglial cells are activated, whereas astrocytes regress. This glial behavior may contribute decisively to the onset and development of neuropathy in the diabetic retina.

The astrocytes play a critical role during normal inner retinal vascularization (22) and degeneration of retinal astrocytes in ischemic tissues is associated with failure of the blood retinal barrier (23). An intravitreal injection of these cells in retinal ischemia not only results in a lack of pathological neovascularization, but also in rapid revascularization of the areas with vessel occlusion (24). An intravitreal injection of these cells appears to be a better option than anti-VEGF treatment, because the majority

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of astrocytes remain in the vitreous body and release long-term factors responsible for the observed beneficial effects. Treatment by blocking VEGF not only inhibits endothelial cell proliferation and reduces vascular permeability, but also leads to a loss of astrocytes with long-term repercussions, which include development of avascular areas with the consequences they entail. The methods developed to target and protect the glial cells may provide a novel strategy, which facilitates normalized revascularization and prevents the consequences of abnormal neovascularization in retinal vascular diseases.

The deficiency of the accompanying glial reaction in diabetic maculopathy may give evidence of an acute onset of ischemia of the retina, when it comes to irreversible damage of the neurons and glial cells. The natural evolution of changes leads to the disappearance of retinal layers and the formation of areas with no capillary perfusion. Numerous microaneurysms develop near such areas, they are the source of focal leakage, and with deepening hypoxia they cause development of vascular proliferative retinopathy. By contrast, the chronic progressive deterioration of diabetic retinal vessels triggers a cascade of changes in the immediate vicinity of blood vessels. It is assumed that the ability of Müller cells and astrocytes to buffer variations of the blood retina leakage conditioned by the physiological status of RPE, determines the clinical picture of the retinal disease. Diabatic retinopathy comes to the edematous form due to the insufficiency of Müller cells or to traction maculopathy when there is an excessive proliferative reaction. It is believed that epiretinal membranes are initially comprised from only glial components, which are derived from Müller cells and astrocytes (25-27). The processes of these cells may invade through the internal limiting membrane of the retina to the vitreous causing a bond with vitreous collagen and forming the vitreoretinal adhesion and pathological posterior detachment of vitreous. While the epiretinal membrane components that mediate their contractile properties may be hialocytes. Posterior vitreous cortex, which is structurally lamellar, also contains these cells. Hialocytes are mononuclear phagocytes embedded about 50 μm from the internal limiting membrane of the retina. They are the sentinel cells that can stimulate the migration and proliferation of monocytes from the systemic circulation and glial cells from the retina and shrinkage of the vitreous collagen (28,29). Anomalous posterior viteous detachment (APVD) may have different clinical manifestations depending on where the vitreous gel is liquefied and if there are strong vitreoretinal connections. At the periphery, it results in retinal tears and detachments. In the macula, APVD causes vitreo-macular traction syndrome, which results in diffuse diabetic macular edema. At the optic disc and retina, APVD causes vitreo-papillary traction (Fig. 6) and creates a scaffold for the proliferation of vessels in proliferative retinopathy (Fig. 7). If vitreoschisis is present, a place of dissection is crucial. If break occurs in front of the hialocytes remaining on the retinal surface, the vitreous layer is thick and has a large number of cells. It easily shrinks concentrically, which results in the formation of a macular pucker syndrome (Fig. 8). If the break runs backwards from the hyalocytes level, only a thin, delicate membrane remains on the central retina. If it is attached to the optic disc and/or temporal vascular arcades, centrifugal stresses arise, causing macular hole formation accompanied with surrounded retinal edema (Fig. 9). Proliferation of the Müller cell processes within the retina combined with the recruitment of contractile cells on the surface can cause focal neurosensory retinal detachment.

Fig. 6. Vitreopapillary traction syndrome with optic nerve head edema (own material).
Ryc. 6. Zespół trakcji szklistkowo-tarczowej z obrzękiem tarczy nerwu wzrokowego (materiał własny).

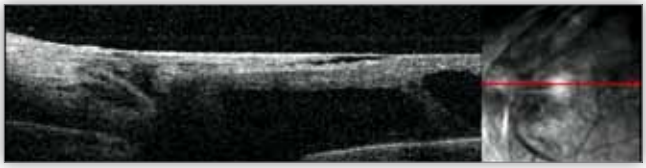

Fig. 7. Fibrovascular proliferations on the optic nerve head and throught temporal superior arcades with tractional retionschisis (own material).
Ryc. 7. Proliferacje włóknisto-naczyniowe na tarczy nerwu wzrokowego i wzdłuż górnych arkad skroniowych z trakcyjnym rozwarstwieniem siatkówki (materiał własny).

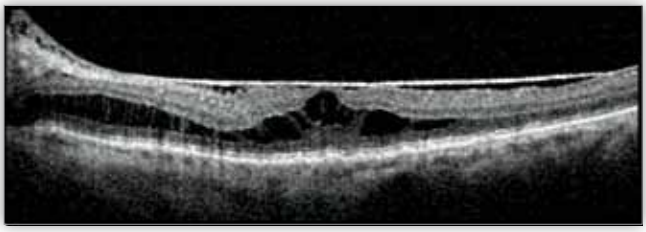

Fig. 8. Epimacular membrane with macular edema (own material).
Ryc. 8. Błona nasiatkówkowa z obrzękiem plamki (materiał własny).

Fig. 9. Full-thickness macular hole formation during long-lasting diabetic macular edema with epiretinal membrane (own material).
Ryc. 9. Pełnościenny otwór plamki w przebiegu długo trwającego cukrzycowego obrzęku plamki z błoną nasiatkówkową (materiał własny).

Conclusions

The key to helping patients with diabetic maculopathy is the search for risk factors in diabetic retinopathy progression (including chronic hypertension, or hyperglycemia), their accurate diagnosis (24-hour blood pressure monitoring and testing of HbA1c level), and proper classification for treatment based on OCT and fluorescein angiography. A comprehensive assessment of the central retinal circulatory disorders including choroidal and retinal circulation in conjunction with proper evaluation of vitreomacular status allows for selecting an appropriate therapeutic algorithm.

It seems that treatment should be started early when there are no irreversible changes in the external layers of the retina. If a laser procedure (peripheral leakage) is possible it could be the first line of treatment. A complete vitrectomy with the removal of ILM should be quickly performed if the photocoagulation pro-

cedure fails. This method is safe and if carried out early creates great opportunities for the mobilization of repair processes within the glial cells in the macula and preserves visual function in patients with diabetic maculopathy. By removing ILM we obtain the effect of reactive gliosis, which allows for maintaining the blood-retina barrier and prevents the progression of diabetic maculopathy. In the future we may supply the recombinant factor from the retinal pigment epithelium PEDF. That method may induce a more physiological Müller cells morphogenesis, with restoration of their connections to photoreceptors and prevent its degeneration, thus improving vision functions. Treatment based on the supply of intravitreal astrocytes appears to be a promising future strategy for treatment of vascular diseases of the eye.

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