Diabetic Choroidopathy in the Pathogenesis of Diabetic Retinopathy

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Contributor: Luca Scuderi , Serena Fragiotta , Mariachiara Di Pippo , Solmaz Abdolrahimzadeh

Diabetic microangiopathy is one of the main responsible factors of multiorgan complications in diabetes, including nephropathy, diabetic retinopathy (DR), cardiovascular disease, and neuropathy. The retinal vascular system, neurons, and glia constitute the neurovascular unit (NVU), where these structures present a close interdependency that promotes autoregulation, maintains the blood–retinal barrier (BRB), and provides structural support. Diabetes affects the NVU, leading to retinal dysfunction and microvascular damage. An early loss of neurovascular coupling, neurodegeneration, glial alteration, and neuroinflammation can occur even before the microvascular alterations become appreciable. Although the retinal microvasculopathy in DR is preponderant and has captured most of the attention in clinical studies, the choroidal vascular layer changes are not fully elucidated. The choroidal vascular bed nourishes the outer retinal layers; the foveola, in particular, relies solely on the choroid.

diabetic choroidopathy diabetes mellitus diabetic retinopathy choriocapillaris choroid optical coherence tomography

1. Structural Organization and Molecular Characteristics of the Choroid

1.1. Anatomical and Functional Organization of the Choroid

The choroidal vascular layer is supplied by the posterior ciliary arteries (PCAs), branches of the ophthalmic artery [1]. PCAs divide into branches that enter the sclera, lateral, medial, or less frequently, superior to the optic nerve. During their intraorbital course, PCAs divide into branches known as long and short ciliary arteries that do not directly arise from the ophthalmic artery and thus need to be differentiated from PCAs [2]. Long posterior ciliary arteries (medial and lateral) run radially in the horizontal meridian to reach the iris. Short posterior ciliary arteries (around 6 to 12) and the perpendicular terminal arterioles supply the innermost portion of the choroid, the CC [3][4]. More in detail, the choroid presents different vascular regions of different calibers, which include, from the innermost to the outermost layers, choriocapillaris (CC), Sattler's, and Haller's layer [5]. The CC is constituted of a capillary bed of fenestrated endothelial cells underneath *BrM*; the fenestration is mostly on the retinal side to allow the transit of nutrients to the RPE and the photoreceptors, but also the removal of waste products through the systemic circulation [6]. Sattler's vascular layer is constituted of medium to small arteries, arterioles feeding the capillary layer, and veins, while Haller's vascular layer contains large-diameter vessels of arteries and veins [5][7].

The lateral and medial PCAs supply the corresponding choroidal regions, with the medial PCA supplying the nasal choroid up to the fovea, often including the optic nerve, and the lateral PCA supplying the remaining choroidal area [1]. Each short PCA supplies a choroidal sector from the posterior pole to the equator [2], while long PCAs extend radially in the horizontal meridian, supplying small peripheral choroidal sectors posterior to the equator on the nasal and temporal sides [2]. Given its segmental nature, a compromised PCA flow can affect half of the choroidal circulation [2].

The CC has a predominant segmental organization with watershed zones, which present a particular vulnerability to ischemia or anoxia [4]. In vivo angiography studies demonstrated the CC organization in functional units characterized by lobules with a terminal arteriole and venous drainage, which do not anastomose with neighboring lobules [1][4]. The CC is more compact in the posterior pole, becoming less dense towards the periphery [2]. The venous drainage of the choroid is granted by vortex veins that exit the globe [2].

Physiologically, the choroidal circulation provides 80% of the oxygen consumed by the photoreceptors, while the remaining 20% is supplied by the retinal vasculature [8][9].

1.2. Immunological and Molecular Composition in the Choroidal Vasculature

The choroid is a highly active immunological site with resident inflammatory cells and continuous trafficking between the systemic circulation and the choroidal stroma [10][11]. The immune cell trafficking is mediated by a high expression of adhesion molecules at the choroidal level, which enable endothelial anchoring and stromal invasion for immunosurveillance [11][12]. CC fenestrated endothelial cells express vascular endothelial growth factors (VEGFs) 1- and 2- receptors. The CC also constitutively expresses intracellular adhesion molecule-1 (ICAM-1) that mediates the leukocytes adhesion via CD11a/CD18 or CD11b/CD18 integrins on endothelial cells [13][14]. ICAM-1, a glycoprotein that is typically expressed on endothelial cells, mostly during an immune response, is instead expressed constitutively on endothelial cells of the healthy CC [14][15].

Human leukocyte antigen (HLA) class I proteins are primarily expressed by the endothelial cells of the CC, and only minimally by the large choroidal vessels [16][17]. Different immune cell types were recognized in the choroid. Mast cells were identified in the choroid perivascularly along blood vessels of medium (Sattler's layer) and large caliber (Haller's layer), but preferentially distributed on the vascular walls of Sattler's layer [18][19]. Degranulation of choroidal mast cells can be responsible for remarkable changes in the posterior segment, including choroidal thickening, choroid vessel dilation, and outer retinal barrier breakdown [20]. Resident choroidal macrophages (CD68+) were also identified in normal eyes, but without the expression of inducible nitric oxide synthase (iNOS) in *BrM* and the choroid. The expression of iNOS in activated macrophages during the inflammatory response exhibited cytotoxic and pro-angiogenic activity [21].

The enzyme carbonic anhydrase IV (CA4) was shown to be abundant in the endothelial cells of the CC, but not in the larger choroidal vessels of Sattler's and Haller's layer, making this one of the most specific markers of the CC

[12][22][23]. Under physiologic condtions, CA4 is involved in the tranport of carbon dioxide (CO_2) and ions contributing to the maintainance of the acid–base balance with the conversion of water and bicarbonate [22].

2. Pathogenetic Aspects of Choroidal Microvasculopathy in Diabetic Retina

2.1. Histopathological Changes

The CC represents the innermost vascular layer of the choroid, constituted of a capillary bed beneath *BrM* [5][24]. This vascular layer presents wide lumens and fenestrated endothelium surrounded by a thin basement membrane, which becomes focally or diffusely thickened in diabetic eyes. The basement membrane thickening mainly affects the scleral side of the CC close to pericytes [25]. The prominent PAS-positive basement membrane material tends to narrow and obliterate the capillary lumens throughout the choroid, with or without fibrosis. In this context, hypertrophy and hyperplasia of the endothelial cells are rare findings [25][26]. Eosinophilic PAS-positive nodules are also evident at the posterior pole and juxtapapillary region, originating from an excessive accumulation of abnormal basement membrane (BM) [25].

Two main patterns of CC degeneration are recognized using alkaline phosphatase (APase) enzyme histochemical activity. A diffuse pattern is characterized by the involvement of capillary segments without defined areas of absolute CC loss and a complete loss with a defined border of CC atrophy [26][27][28].

Capillary dropout is evident at any stage of DR, even in eyes without retinopathy or mild signs, with a topographical predilection from the temporal equator to the periphery. Capillaries with a beaded morphology and tortuosity are common features in diabetic choroidopathy [27][29]. Combining their findings with the existing literature, Cao et al. hypothesized that diabetic choroidopathy could be defined as PAS-positive material accumulation within the intracapillary stroma, often accompanied by wart-like extrusions on the vascular lumen [26].

2.2. The Role of Inflammation in Diabetic Retinopathy

Diabetes mellitus exhibited an inflammatory basis with increased tumor necrosis factor α (TNF-α), a pleiotropic cytokine playing a critical role in the inflammatory process and disease progression [30]. The thickening of capillary BM has been considered one of the most important events driving the microvascular damage in DR [31]. The deposition of extracellular matrix (ECM) constituents, including collagen IV, fibronectin, and laminin, contributes to the development of a thicker BM [31][32]. An altered ECM remains an early and crucial event in diabetic retinopathy (DR), leading to fibrosis and increased vascular stiffness and permeability [33]. The alterations at the ECM level have demonstrated to influence the leukocyte adhesion leading to leukostasis [34][35]. Inflammatory cytokines proven to induce significant changes in the expression of ECM constituents included TNF-α and IL1-β. TNF-α significantly modified the expression of collagen IV, fibronectin, agrin, and perlecan, while IL1-β influenced the expression of collagen IV and agrin [36].

Several inflammatory cytokines have been implicated in DR, including interleukins 1 (IL-1), IL-6, IL-8, TNF-α, and monocyte chemotactic protein-1 (MCP-1) [37][38]. The activation of microglia under the condition of hyperglycemia produced an increased expression of nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB), leading to increased oxidative stress and pro-inflammatory cytokines, such as IL-1b, VEGF and TNF-α, chemokines, and adhesion molecules, including E-selectin and ICAM-1 [37]. The inflammatory response with cytokines activation remains the main one responsible for the microvascular damage and cellular apoptosis in DR [39].

2.3. Molecular Mechanism Underlying the Choriocapillaris Loss

Inflammation appears to be a crucial element in the etiology of CC loss in diabetes. Among the activated leukocytes, polymorphonuclear neutrophils (PMNs) appear to be the leading players in diabetic choroidopathy pathogenesis. These inflammatory cells are increased in number and co-localized with areas of choroidal capillary loss, suggesting a role in the vaso-occlusive events and endothelial damage in the diabetic choroid [40][41]. Hyperglycemia seems to drive the PMN stimulation, which is in an activated state, leading to increased production and release of oxidizing agents and enzymatic exocytosis, contributing to the microvascular changes [42]. Under inflammatory stimuli, PMNs roll along the endothelium before adhering firmly to it. Activated PMNs express CD11/CD18, responsible for adhesion via binding ICAM-1 [40][43]. The interaction between PMN and endothelium is likely mediated by adhesion molecules, such as P-selectin and ICAM-1 [14][44]. At the choroidal level, ICAM-1 is expressed almost constitutively in the CC, while P-selectin is represented in postcapillary venules. The expression of these adhesion molecules is low in the retinal vessels, reflecting the difference between these two vasculatures [14][15].

The production of ICAM-1 in diabetes mellitus is also increased by nonperfusion, ischemia, and elevated TNF-α levels. The high expression of ICAM-1 on endothelial cells and elevated neutrophils represent initiating events for capillary obstruction and nonperfusion, leading to disease progression [14].

A possible explanation for the role of PMNs in producing vaso-occlusive phenomena in the diabetic choroid included the formation of queues of PMNs that could initially reduce the flow until stopping it. The accumulation of PMNs is probably due to a combination of increased leukocyte adhesion molecules and delayed blood flow [41]. The blockage of ICAM-1 via neutralizing antibodies prevents leukostasis and vascular leakage [45]. Likewise, the blockage of CD-18 impedes PMN adhesion and leukostasis [46].

Another pathogenic mechanism for diabetic choroidopathy included choroidal flow regulation. Nitric oxide (NO) is a signaling molecule produced by the vascular endothelium and involved in several physiological functions. NO synthases (NOSs) are a family of enzymes directly responsible for NO production, which include three main isoforms: neuronal NOS (nNOS), inducible NOS (iNOS), and endothelial NOS (eNOS) [47]. The nNOS and eNOS isoforms are directly involved in choroidal blood flow regulation [48]. Choroidal vasodilatation is mediated by the parasympathetic system through vasoactive intestinal polypeptide, NO derived by nNOS and acetylcholine [12]. Immunoreactivity demonstrated a preferential localization for eNOS in the endothelial and smooth muscle cells of

retinal arteries and capillaries, while nNOS was mainly in a subpopulation of amacrine cells. At the choroidal level, eNOS was prominently localized in the CC and less in the large choroidal vessels and stroma, and nNOS in the RPE nuclei, while iNOS was localized in the choroidal blood vessels and stroma [49]. The biological activities of NO and NOS at the choroidal level include the regulation of vascular tone, inducing vasodilatation and proangiogenic activity [49][50]. A possible role of nNOS in diabetic choroidopathy was speculated about after evidence of reduced choroidal nNOS expression 6 weeks after the development of diabetes. Choroidal nNOS is mainly expressed in the parasympathetic perivascular nerve fibers surrounding the choroidal arteries and veins, suggesting that diabetic choroidal microangiopathy can result from this imbalance [51].

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