Chapter

Role of Inflammation in Diabetic Retinopathy

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Abstract

As the global burden of diabetes is increasing there is a corresponding increase in the complications associated with the same. Diabetic retinopathy is a sight threatening complication of diabetes mellitus which was considered to be a microvasculopathy. Recent evidence however, has brought to light that inflammation may be a key player in the pathogenesis of this condition. Levels of inflammatory mediators like Hypoxia inducible factor, TNF-α, IL-6 and IL-1B amongst others have been noted to be elevated in the diabetic vitreous gel. The concept of the neurovascular unit better explains the changes that take place resulting in the breakdown of the blood retinal barriers and how these inflammatory mediators affect the morphology of the retina at a cellular level. Glial cells form a key instrument of this neurovascular structure and are also the cells from where the inflammatory response is initiated. Understanding of the pathogenesis of diabetic retinopathy will help us in finding targeted therapies which may provide long term benefits and possible cure. Few anti-inflammatory medications have shown promise albeit in a small clinical or experimental laboratory setting. However, future research may lead to better understanding of the disease and a better pharmacological intervention.

Keywords: pathogenesis of diabetic retinopathy, glial cells in diabetic retinopathy, retina inflammation, steroids, cytokines

1. Introduction

In the past few years, unhealthy lifestyle coupled with obesity has led to a rampant increase in the global burden of diabetes mellitus. As per WHO the global prevalence of the condition was 422 million in the year (2014) with 8.5% of adults aged more than 18 years suffering from this condition. Approximately 1.6 million deaths yearly are supposed to be caused by diabetes alone, this number excludes the mortality associated with cardiovascular events, renal disease and tuberculosis secondary to chronic hyperglycemia [1]. The global burden of diabetes is expected to swell to 642 million adults by the year 2040 with 75% of the affected individuals belonging to low and middle income countries. Diabetic retinopathy affects 1 in 3 adults with diabetes and is one of the major causes of blindness in the working-age population [2].

Diabetic retinopathy (DR) is a major microvascular complication of diabetes and it is categorised into a non-proliferative stage (NPDR) or proliferative stage (PDR) depending on the presence of retinal microvascular changes. The non-proliferative stage is characterised by the presence of microaneurysms, cotton wool spots, vascular tortuosity, retinal haemorrhage and lipid exudation while in the

proliferative stage aberrant new blood vessels develop which are fragile and can extend into the posterior cortical vitreous [3]. Another important element in the vast conundrum of DR is diabetic macular edema (DME). It can occur across all levels of DR changes and compromises central vision. DME is the most common cause of diminished vision in an individual with DR [4].

2. The neurovascular unit in diabetic retinopathy

In recent years a concept of neurovascular unit in diabetic retinopathy has emerged. This is based on the findings that neurodegeneration is one of the earliest changes in a case of diabetic retinopathy. Indeed, a reduction in oscillatory potential in the electroretinogram is the first measurable change in retinal function, being recorded even in cases wherein there is no clinical change suggestive of DR. This indicates that neurodegeneration precedes microvascular abnormalities [5].

The retinal neurovascular unit includes the physical and biochemical interactions amongst the neurons, the vascular beds and the supporting cells of the retinal framework. The neural unit includes the ganglion cells and the glial cells while the vascular component of the unit is made up of the endothelial cells and the pericytes. The neurovascular unit reflects the inter-dependance of the vascular barrier and blood flow regulation on the glial cells, pericytes and neurons as well as their reciprocal dependance on vascular support. Together with the neurovascular unit the retinal pigment epithelium contributes to the formation of the blood retinal barrier [6]. The inner blood retinal barrier (iBRB) encompasses the endothelial cells of the retinal microvasculature which are covered by astrocytes, pericytes and muller cell end-feet. It regulates the transport across the retinal capillaries and maintain the micro-environment of the inner retina. The outer blood retinal barrier (oBRB) comprises the tight junctions of the neighbouring RPE cells and it serves as a filter for nutrients and solutes from the blood [7].

3. Histopathological changes in diabetic retinopathy

Diabetic retinopathy has been traditionally described as a microvasculopathy, however newer evidence has suggested that inflammation may provide a substantial role in the histopathological changes that are noted in the disease.

3.1 Microvascular changes

Retinal ischemia is the initiator that propels the plethora of changes that occur in DR. In the initial stages, prior to even the development of visually significant vascular alterations, there is a disruption of vascular auto-regulation which leads to oxygen and nutrient deprivation in the inner retinal layers [8].

Retinal vascular basement membrane thickening is present in the early stages of the disease and is mediated by hyperglycemia. Impaired sugar levels lead to an up-regulation of extracellular matrix proteins, collagen and fibronectin [9]. This thickening of the basement membrane may lead to an impairment in cell to cell communication between the endothelium, pericytes, glial cells and the retinal immune cells, ultimately leading to a loss of function [10]. Loss of pericytes leads to a weakening of the capillary wall; this ultimately results in the development of areas of out-pouching labelled as microaneurysms (MA), which are amongst the first clinically detectable signs of DR [11].

A progressive occlusion of the retinal capillaries is noted on histopathological evaluation of post-mortem specimens. Stitt et al. [10] evaluated trypsin digest preparations of these capillaries and noted them to be acellular tubes of naked basement membrane without endothelial cells. The loss of endothelial cells can be attributed to pericyte death which ultimately culminates into breakdown of the inner blood retinal barrier.

The breakdown of the inner blood retinal barrier would lead accumulation of fluid and exudates in the retinal layers contributing to diabetic macular edema (DME). Fluid exuding out from the superficial capillary plexus leads to accumulation of fluid in the inner nuclear layer while that exuding out from the deep capillary plexus is believed to collect in the outer plexiform layer. Cystoid spaces noted in the macula appear due to liquefaction and necrosis of the muller cells and the production of prostaglandins and inflammatory cytokines [12].

3.2 Neuroglial changes

The muller cells, astrocytes and microglial cells are present in close vicinity of the retinal blood vessels and help to maintain retina homeostasis. Muller cells play a central role in the retinal metabolism and hence are susceptible to the metabolic alterations of diabetes. An increased production of glial fibrillary acidic protein (GFAP) by the muller cells is noted in the early part of the disease, which plays a key role in gliosis. This is indicative of a state of glial hypertrophy [13].

Microglial cells are the resident inflammatory cells of the retina which get activated in DR. In the early stages of mild DR a hypertrophy of the microglia is noted and the cells are settled along the retinal plexiform layers. When the DR progresses into a proliferative stage the microglial cells are found to be extensively distributed in areas of retinal ischemia and neovascularisation [14]. A strong tendency of the microglial cells to invade the outer retinal layers is also noted in prolonged DR [15].

3.3 Neuronal changes

Overt degeneration of retinal neurons during diabetes is a concept that was first described in the 1960s in post-mortem patient samples. By the late 1990s experimental evidence reinforced this finding by demonstrating that depletion of some neuronal populations occurred in diabetic rodent models, possibly even prior to appearance of obvious microvascular lesions. Apoptosis of the retinal ganglion cells (RGCs) is histologically noted in diabetic retinas. This also accounts for the diminished thickness of the retinal nerve fibre layer on optical coherence tomography (OCT). It is believed that hyperglycemia induces a down-regulation of neuronal growth factors thereby contributing to programmed cell death [16].

3.4 Immune cell activation

Histological evaluation of blood vessels, early in the course of DR, have shown increased interaction between leukocytes and endothelial cells. This phenomenon called leukostasis is characterised by an adherence of monocytes and neutrophils to the endothelial lining of the blood vessels. This leads to blockage of the thin retinal capillaries and areas of retinal non-perfusion [17].

3.5 Retinal pigment epithelium and choroid

The changes are not only localised to the inner retinal layers but also extend to the RPE and the choroid compromising the outer blood retinal barrier. RPE

dysfunction and leakage from the choriocapillaris is noted leading to outer retinal edema and impaired clearance of fluid [18].

Choroidal atrophy has been noted in cases of long standing DR, with a diminished choroidal thickness noted on OCT evaluation. This thinning of the choroid have been linked to HbA1c levels and may lead to choroidal neovascular membranes or intra-choroidal microvascular abnormalities [19].

4. Inflammation in DR

4.1 What is inflammation?

Inflammation is non-specific response of the the body to injury or stress which includes a variety of molecular and cellular mediators. Tissue stress may lead to de-inhibition of the transcription factor nuclear factor kappa beta (NF-κB) which stimulates the production of acute phase proteins, pro-inflammatory cytokines and chemokines such as TNF-α, IL-6 and IL-1B amongst others. These pro-inflammatory mediators play a major role in the unfolding of the inflammatory processes with recruitment and activation of monocytes and leukocytes. Inflammation usually resolves spontaneously in a coordinated manner, however when this fails to happen the beneficial effect of inflammation is lost and consequences ensue.

4.2 Inflammation in pathogenesis of diabetic retinopathy

If we consider diabetic retinopathy to be a disease mediated via the inflammatory pathway then anti-inflammatory medications should provide some degree of safety. This was noted in 1964 by Powell and Field who reported that patients with rheumatoid arthritis on high dose aspirin therapy tended to have a less severe form of DR [20]. Histological features of DR were less commonly noted in dogs wherein aspirin was initiated in a dose of 20-25 mg/kg/day shortly after the diagnosis of diabetes mellitus and continued for a period of 5 years [21].

Increased concentrations of inflammatory cytokines such as IL-1, IL-6, IL-8, TNF-α and MCP-1—have been reported in ocular tissues from non-proliferative DR (NPDR) patients. The accumulation of these cytokines is believed to lead to early neuronal cell death. Cytokines such as MIP-1, IL-3 and IL-1 are believed to have a role in the angiogenesis. Thus inflammation may contribute towards and precede the development of neovascularisation [22]. Cylco-oxygenase-2 (COX-2) is expressed in the retinal astrocytes in human diabetic retinas. Prostanoids generated from COX-2 lead to an increased expression of VEGF and other pro-angiogenic factors, thereby contributing to development of proliferative diabetic retinopathy [23].

In case of diabetic macular edema (DME) levels of pro-inflammatory molecules Vascular Endothelial Growth Factor (VEGF) and IL-6 are noted to be elevated as per different studies. In particular DME associated with sub-retinal fluid on OCT shows elevated levels of these cytokines [24].

4.3 Diabetes and inflammation

The signal for the initiation of inflammation in a diabetic retina is believed to be metabolic in origin. Cell death was proposed as one of the causes, however, retinal cell death in DR is primarily via apoptosis and hence may not be associated with an inflammatory response. Certain factors that contribute directly or indirectly to increased inflammation are summarised below:

4.3.1 Hyperglycemia

Presence of hyperglycemia is linked with a pro-inflammatory environment. Retinal cells when incubated in high glucose environment led to increased production of iNOS, COX-2 and leukotrienes [25]. Furthermore, Joussen et al. in 2004 demonstrated diabetic retinopathy like disease following a sugar rich diet in laboratory mice. This was associated with leukostasis and increased vascular permeability [26].

4.3.2 Oxidative stress

Diabetes is known to produce oxidative stress at a molecular level. Two months of diabetes in rats led to a significant increase in levels of IL-1 and NF-κB. This increase is inhibited by antioxidants. It is believed that oxidative stress induced increase in retinal permeability and inflammation is mediated via the WNT signalling pathway [27].

4.3.3 Lipids

Diabetes results in a decrease in the levels of poly-unsaturated fatty acids especially docosohexanoic acid (DHA) and these changes are associated with chronic inflammation. Long term administration of omega 3 fatty acids has been linked to retinal capillary degeneration. DHA, resolvins and autocoids have shown to have critical anti-inflammatory properties. Li et al. noted that administration of statins (HMG-CoA inhibitor) inhibited diabetes induced changes in the blood retinal barrier [28].

4.3.4 Age

Interaction between the advanced glycation end-products (AGE) with its receptor (RAGE) is known to have pro-inflammatory consequences. Pharmacological inhibition of RAGE signalling led to a significant decrease in retinal capillary degeneration and other early lesion of DR in animal models [29].

4.3.5 Hypertension

Hypertension is a main secondary risk factor associated with DR. Silva et al. [30] in 2007 found an increased expression of VEGF and ICAM-1 in an experimental model in rats who were in a prehypertensive or hypertensive group. They concluded that hypertension led to an increased inflammatory response in the diabetic retina and consequently worsened retinopathy.

4.4 Inflammatory mediators involved

A variety of factors are involved in the cascade of the inflammatory process in a diabetic retina. It has been seen that intravitreal levels of cytokines, chemokines and growth factors change under inflammation leading to increased secretion from endothelial cell and development of neovascularization. The major factors are highlighted below:

4.4.1 Growth factors

4.4.1.1 Vascular endothelial growth factor (VEGF)

It is known that VEGF is the principal target of pharmacologic intervention for proliferative diabetic retinopathy and studies have confirmed elevated levels

of VEGF in vitreous samples in DR causing increased vascular permeability [31, 32], stimulates angiogenesis because of its mitogenic effect on endothelial cells, and enhances endothelial cell migration and survival [33, 34] and their production increases markedly under conditions of hypoxia [35]. Intercellular adhesion molecule-1 (ICAM-1) is involved in inflammation and acts as a local intensifying signal in the pathological processes associated with chronic eye inflammation. It has been seen that VEGF increases ICAM-1 and leukocyte adhesion to vessel wall and elevated ICAM-1 and cell adhesion molecule-1 synthesis in retina. It further increases ICAM-1 in endothelial cells and this in turn leads to activation and increased production of cytokines and leukocyte activation [36], these cytokines initiate and mediate the inflammatory response and stimulate further release of VEGF [37]. Various studies have strongly indicated that the increased level of ICAM-1 generally exists in the patients with DR and it may associated with the severity of DR [38]. Placental growth factor (PGF), member of VEGF family, binds to VEGF- and neuropilin-receptor sub-types. PGF induces a range of neural, glial and vascular cell responses that are distinct from VEGF-A. As its expression is associated with pathological angiogenesis and inflammation, its blockade does not affect the healthy vasculature [39]. High levels of PGF have been found in aqueous humour, vitreous and in retina of patients especially those with diabetic retinopathy (DR). Results suggest that anti-PGF therapy might have advantages over anti-VEGF treatment, and that it may have clinical applications as a standalone treatment or in combination with anti-VEGF [39]. Low concentrations of VEGF have been seen to rise with PGF stimulating endothelial cell proliferation, migration, and angiogenesis [40]. Higher levels of PGF in vitreous are seen in DR and these levels are correlated well with VEGF levels [41].

4.4.1.2 Tenascin-C (TNC)

Tenascin-C is an extracellular matrix protein and plays an important role in cell growth and adhesion, playing an equally involved in angiogenesis, oncogenesis, wound repair and inlflammation [42, 43]. Studies have shown that TNC is involved in the pathogenesis of ischemic proliferative retinopathy. Elevated levels have been detected in PDR vitreous humour. mRNA and protein expression of TNC has been found in pre-retinal fibrovascular membranes excised from PDR patients [44]. Extracellular matrix (ECM) synthesis plays an important part in the pathogenesis of the intravitreal membranes and is thus characteristic of both proliferative vitreo-retinopathy (PVR) and early stages of proliferative diabetic retinopathy (PDR). Hence it is clear that TNC plays a role in the development of epiretinal PVR and PDR membranes by controlling cell adhesion and regulating extracellular matrix formation formation [45].

4.4.1.3 Insulin like growth factor

It is known that IGF-1 is has a significant role in pathogenesis of DR as it is involved in regulation along with influencing growth, maturation and functioning of blood vessels. It also activates VEGF in human RPE cell and receptors which are actively involved in development of vitreo-retinal disorders [46, 47]. Insulin-like growth factor-I (IGF-I) is known to enhances insulin action in normal subjects and in both type 1 and 2 diabetes. It is associated with significant side effects in a high percentage of patients. Simultaneous administration of IGF binding protein-3 with IGF-I limits IGF-I inducible side effects, but it does not downgrade the ability of IGF-I to enhance protein synthesis and bone accretion [48]. Severity of DR in patients with

type 1 diabetes is inversely related to serum IGF-1 levels. Low IGF levels are an indicator for closer follow-up and strict management of diabetes and retinopathy [49].

4.4.1.4 Basic fibroblast growth factor

Amongst the factors which play a role in mitogen and antigenic activity involving survival and maturation of glial cells and neuron, basic fibroblast growth factor (bFGF) play an important role [50]. Neurotrophic factors synthesised from glial cell line stimulates Muller cells which produce bFGF, which initiates endothelial cell proliferation and VEGF production [51, 52]. Studies have detected presence of two growth factors in same cells of ocular neovascular membrane suggesting more than one growth factor may contribute to defective angiogenesis. Growth factors are not exclusively seen in neovascular tissues and are not localised mainly in the vascular endothelium as shown by this study which detected their presence in choroidal neovascular membranes also [53]. Another study documented increased levels of basic fibroblast growth factor in vitreous specimens from patients with proliferative diabetic retinopathy, particularly those with active proliferative retinopathy [54]. Various studies have shown that bFGF, nerve growth factor, and glial cell line-derived neurotrophic factor are also part of process involved in the formation of epiretinal membranes in PDR [55]. This confirms that both vegf and basic fibroblast growth factor are present in diabetic eyes and part of process causing PDR.

4.4.1.5 Aminopeptidase

Adipocytes are involved in production of a polypeptide hormone named aminopeptidase, which circulates at very high levels in the bloodstream, exerts anti-inflammatory effect. It also expresses an anti-atherosclerotic effect and inhibits intimal thickening and vascular smooth muscle cell proliferation in injured arteries [56]. Angiogenesis, a neo-vessel formation from pre-existing micro-vessels requires sequential steps involving detachment of pre-existing pericytes for vascular destabilisation, extracellular matrix turnover, migration, proliferation, tube formation by endothelial cells, and reattachment of pericytes for vascular stabilisation. Aminopeptidases has been found to regulate the N-terminal modification of proteins and peptides for maturation, activation or degradation, and thereby relate to a variety of biological processes. Three types of aminopeptidases which have been reported are involved in angiogenesis. They include type 2 methionine aminopeptidase, aminopeptidase N, and adipocyte-derived leucine aminopeptidase/puromycin insensitive leucyl-specific aminopeptidase [57]. It has been documented and shown by Costagliola et al. [58] that APN levels in aqueous humour of patients with type 2 diabetes, PDR, and macular edema are higher than in aqueous of control subjects.

4.4.1.6 Connective tissue growth factor

Connective tissue growth factor, also known as CCN2, is a cysteine-rich matricellular protein forms part of control on biological processes, such as cell proliferation, differentiation, adhesion and angiogenesis, as well as multiple pathologies, such as tumour development and tissue fibrosis [59]. Possible role of CTGF, CD105, and gelatinase B in the pathogenesis of proliferative vitreo-retinal disorders has been suggested by various studies [60]. It has been see that both CTGF and VEGF levels are elevated in PDR patients and CTGF could help in development of proliferative membranes in PDR though plays no role in retinal neovascularization [61].

4.4.1.7 Hepatocyte growth factor

Its has been seen that HCG and its receptor unit control motility, growth and morphogenesis of various cell types and possess angiogenic activity [62]. Data indicates that HGF is a pro-permeability, pro-inflammatory, and pro-angiogenic factor and along with its activator is found increased in ischemic retina providing support for a potential role of HGF in macular edema and in ischemic retinopathies such as diabetic retinopathy [63]. Elevated levels of HCG in aqueous have been found to be directly related to degree of PDR [64].

4.4.1.8 Stem cell factor

EPO is a glycoprotein which is multifunctional, produced in foetal liver and adult kidney when exposed to hypoxic conditions [65]. It possess anti-inflammatory, antioxidant, pro-angiogenic properties [66–68]. Some studies have also documented neuro-protective and anti-apoptotic properties [69, 70]. The mechanisms controlling the expression of the gene encoding for the hormone erythropoietin (EPO) are exemplary for oxygen-regulated gene expression. In humans and other mammals, hypoxia modulates EPO levels by increasing expression of the EPO gene [71]. Expression of EPO is mediated by HIF-1α which simultaneously stimulates VEGF secretion [72]. The hormone erythropoietin (EPO) maintains red blood cell mass by promoting the survival, proliferation and differentiation of erythrocytic progenitors. Circulating EPO originates mainly from fibroblasts in the renal cortex. EPO production is controlled at the transcriptional level. Hypoxia attenuates the inhibition of the EPO promoter by GATA-2 [73]. In patients with PDR, both EPO and VEGF are up-regulated into the vitreous each acting independently [74]. It has been seen that inhibition of EPO or VEGF leads to suppression of retinal neovascularization, results are best when both are suppressed together and in vitro inhibition of EPO leads to attenuation of endothelial cell proliferation in PDR [75].

4.4.2 Transcription factors

4.4.2.1 Nuclear factor kappa beta (NF-κB)

NF-κB is a pro inflammatory transcription factor and a regulator of inflammation related to immune responses, cellular proliferation and cell apoptosis [76]. It is located in endothelial and retinal pericytes and released on exposure to hypoxia and hyperglycemia and thereafter releases cytokines, chemokines, and other pro-inflammatory molecules [59]. Once NF-κB is activated it leads to production of cytokines, chemokines and other pro-inflammatory molecules [77]. Studies have shown a relation between NF-κB activation and downstream up-regulation of vascular endothelial growth factor (VEGF) in DR. VEGF SNPs i.e., RS2010963 C allele and RS3025039 T allele might be strongly associated with PDR occurrence and in turn regulating VEGF expression in PDR subjects [78]. NF-κB is also involved in the formation of both glial and vascular endothelial cellular components, and that these two cell types might have functional interactions that lead to the enlargement of intraocular proliferative membranes namely ERM [79]. Receptor activator of NF-κB ligand (RANKL) is a member of the tumour necrosis factor (TNF) superfamily. RANKL increases endothelial permeability and induces angiogenesis, suggesting its critical roles in the vasculature. Hence the use of an RANKL blockade as a potential therapeutic approach against ischemic retinopathies is confirmed [80]. It is important to remember that the signal related to RANKL plays a role in

the pathogenesis of insulin resistance and suggests a link between inflammation and the pathogenesis of type 2 diabetes mellitus [81].

4.4.2.2 Hypoxia inducible factor

Adapting to hypoxic conditions leads to cellular and tissue transcriptional induction involving genes that participate in angiogenesis, glucose metabolism, and cell proliferation and survival. The principal factor mediating this response is the hypoxia-inducible factor-1 (HIF-1), an oxygen-sensitive transcriptional activator. It consists of a constitutively expressed subunit HIF-1β and an oxygen-regulated subunit HIF-1α [82]. It has been shown that diabetic factors result in HIF-1 production and angiogenesis and Treins et al. [83] showed that insulin-like growth factor-1 (IGF-1) stimulates accumulation of HIF-1 in human retinal pigment epithelial cells. As HIF-1 becomes active it activates several genes, including the genes for IL-6, IL-8, and pro-angiogenic growth factors. It has also been shown that acute intensive insulin therapy exacerbates the diabetic induced BRB breakdown through HIF-1 and VEGF [84] and presence of HIF-1α has been demonstrated in diabetic epiretinal membrane [85]. NF-κB controls the expression and synthesis of HIF-1 in response to inflammatory stimuli. Hypoxia activates NF-κB that binds promoter of HIF-1, stimulates the production of IL-6 and IL-8 in the vitreous of patients with PDR. HIF-1α, Ang-2 and VEGF seem to play an important role in the pathogenesis of PDR and simultaneously provide adverse angiogenic milieu in PDR epiretinal membranes favouring aberrant neovascularisation and endothelial abnormalities [84].

4.4.3 Cytokines

4.4.3.1 Interleukin-6 (IL-6)

IL-6 is a cytokine regulates the expression of matrix metalloproteinases (MMPs) which is a primary constituent of the vitreous [86, 87] and it also regulates immune response, increases permeability of vessels and initiates angiogenesis [88, 89]. Studies, have indicated that IL-8, VEGF-A, and PlGF demonstrated a strong correlation in vitreous and aqueous of patients with PDR. The aqueous may serve as a proxy for vitreous for some cytokines involved in PDR. More recently anti-VEGF injections have been able to decrease VEGF-A levels in aqueous, however they did not significantly affect other cytokines, indicating a need for other targeted therapies in PDR management [90]. Role of IL-6 in neovascularization, a key clinical feature of DR, is shown in studies that show IL-6 can not only promote angiogenesis directly but support angiogenesis by inducing expression of VEGF, an angiogenic factor [91]. Hence, the role of IL-6 and IL-8 as angiogenic and factor for causing neovascularization is supported.

4.4.3.2 IL-1β

Macrophages produce IL-1β which is an inflammatory cytokine mainly which can further activate the transcriptional factor NF-κB, which plays an important role in transcription of inflammatory cytokines [92]. Furthermore TNF-α and recombinant IL-1β seem to stimulate human retinal pigment epithelium cells leading to secretion of IL-6 and IL-8 [93]. It has been seen in studies that TNF-α and IL-1β promote angiogenic activity leading to stimulation and synthesis of collagen glial cells, and fibroblasts leading to proliferation and contraction promoting angiogenesis and ocular neovascularization [94]. It has been observed that invading microorganisms activate the inflammatory response by secreting pro-inflammatory

cytokines particularly IL-1β. IL-1-responsive genes initiate and coordinate local inflammation and also attract and activate cells of the adaptive immune system at sites of infection eventually leading these signals to activate NALP3-inflammasome pathway, which plays a central role in acute and chronic sterile inflammation [95]. Studies assessing inflammatory mechanisms involving NLRP3 inflammasome were carried out using akimbo mouse, revealing an increased vascular leakage, reduced retinal thickness, and function in Akimba retina. High levels of IL-1β along with increased NLRP3, ASC, and Caspase-1 at mRNA and protein levels were seen suggesting a critical role for NLRP3 inflammasome in akimbo retina depicting advanced stages of DR pathogenesis [96]. Other studies have shown elevated levels in aqueous of IL-6 and macular thickness indicating IL-6 may play a central role in the development of diabetic macular edema [97].

4.4.3.3 TNF-alpha

TNF-α, a cytokine with tumour necrosis activity is produced by various types of cells which includes macrophages, is recognised as an important host defence factor that affects malignant and normal cells. It is synthesised by T cells and macrophages and its expression is regulated by NF-κB [98]. It also plays the role of inflammatory mediator of neuronal cell after cerebral ischemic trauma and also a similar role in retinal tissue [99]. Besides increasing endothelial cell permeability [100]. TNF-α is also involved in stimulating leukocyte adhesion and inducing oxidation and simultaneous production of reactive oxygen due to the death of retinal ganglion cells and degeneration of the optic nerve [101]. High pharmacological doses of TNF-α combined with chemotherapy has been seen to regress intractable tumours. Evidence demonstrates that pathophysiological concentrations of endogenous TNF-α could act to promote tumour genesis and growth [102]. In diabetic retinopathy pro-inflammatory mediators regulated by cytokines, such as TNF-α, IL-1β and growth factors leads to further progression of these processes, leading to vaso-permeability (diabetes macular edema) and/or pathological angiogenesis (proliferative diabetic retinopathy) [103]. Diabetic patients have shown higher TNF-α levels in vitreous/serum ratio compared to non-diabetics [104]. Strong correlation between plasma TNF-α levels and severity of DR has been documented [105]. It has been documented that TNF-α is expressed in the endothelial cells and stromal cells of the fibrovascular membranes of diabetic patients with PDR [106]. Studies have confirmed the presence of vascular endothelial growth factor (VEGF) and TNF-α in epiretinal membranes in proliferative eye disease [107]. Recent studies assessing the impact of anti-TNF agents on intermediary metabolism suggest that TNF-α blockade could improve insulin resistance and lipid profiles in patients with chronic inflammatory disease [108].

4.4.3.4 HMGB1

HMGB1 though secreted from numerous sites in the retina, including the ganglion cell layer, inner nuclear layer, outer nuclear layer, inner and outer segment of the photoreceptors, and retinal pigment epithelial cells [109, 110]. It is a protein that stabilises the formation of nucleosomes and gene transcription [111]. Studies indicate that HMGB1 is released from activated innate immune cells or necrotic cells and functions as an important mediator of endotoxaemia, sepsis, arthritis, and local inflammation hence agents that inhibit HMGB1 release or action, confer significant protection against endotoxaemia, sepsis, and arthritis in animal models and thus hold potential for the clinical management of various inflammatory diseases [112]. HMGB1 functions as a cytokine that amplifies the effect of the receptor for AGE

(RAGE) axis and mediates the secretion of survival factors such as VEGF-A, to counteract the effects of oxidative stress. HMGB1 is thought to contribute to the accelerated micro and macro-vasculopathy seen in diabetes [113]. Its level has been detected on higher side in vitreous in patients with PDR and has been detected in endothelial and stromal cells of ERM in PDR patients [114].

4.4.4 Chemokines

Chemokines constitute a family of chemoattractant cytokines and are subdivided into four families on the basis of the number and spacing of the conserved cysteine residues in the N-terminus of the protein. They seem to play a role in selectively recruiting monocytes, neutrophils, and lymphocytes, along with inducing chemotaxis through the activation of G-protein-coupled receptors.

4.4.4.1 Monocyte chemoattractant protein-1 (MCP-1/CCL2)

Monocyte chemoattractant protein-1 (MCP-1/CCL2) is considered one of the key chemokines that regulate migration and infiltration of monocytes/macrophages [115]. The expression of MCP-1 is regulated by NF-κB and MCP-1 can induce VEGF production [116]. Both CCL2 and its receptor CCR2 have been demonstrated to be induced and involved in various diseases [115]. Diabetic patients have shown elevated levels of MCP-1 in vitreous and its levels are higher in the vitreous than in the serum indicating local production of MCP-1 [117]. Studies have shown that there is a significant association between the vitreous MCP-1 levels and DR severity [118]. The MCP-1 is also a potent chemotactic factor for monocytes and macrophages that can stimulate them to produce superoxide and other mediators. Following hyperglycemia, retinal pigment epithelial (RPE) cells, endothelial cells, and Müller's glial cells are of utmost importance for MCP-1 production, and vitreous MCP-1 levels rise in patients with DR. Increased expression of the MCP-1 in the eyes can also play a significant role in the pathogenesis of DR [119]. Interferon-gamma inducible protein 10 (IP-10) is a CXC chemokine that is expressed at higher levels in the vitreous of diabetic patients [120], and its vitreous levels are higher than its serum levels [121]. Anti-inflammatory cytokines such as IL-10 and IL-13 may be involved more in the pathogenesis of DR and CRVO than in other diseases and both cytokines and chemokines may be correlated to VEGF in the vitreous fluid and the inflammatory reaction may be more active in CRVO than in DR [122].

4.4.4.2 Monokine

Monokine which is induced by interferon-gamma (MIG) is a chemoattractant for activated T cells and also has angiostatic activity [123]. In their study Wakabayashi et al. reported that MIG could play an role in the pathogenesis of DR and works in cooperation of VEGF in the progression of pathological angiogenesis in DR. The authors have detected higher levels of MIG in vitreous of DR patients [123]. Elevated MIG levels are could be in response to the up-regulation of angiogenic factors such as VEGF. The alternative mechanism could be in play in DR which results in chemotaxis of leukocytes rather than in carrying out its angiostatic functions [26].

4.4.4.3 Stromal cell-derived factor-1

Stromal cell-derived factor-1 (SDF-1) is a chemokine that is up-regulated in response to tissue damage and is involved in stimulation and mobilisation of cells

involved in tissue repair and cellular migration, differentiation, and proliferation of endothelial progenitor cells [124]. SDF-1 repairs after ischemic injury by binding to its receptor, CXCR4 and recruits the progenitors of endothelial cells from the bone marrow. The levels off SDF-1 in vitreous have been found to be on higher side in DME and PDR patients [125]. Studies have demonstrated that inhibiting the N-(carboxymethyl)lysine-induced TPL2/ATF4/SDF1 axis can effectively prevent diabetes mellitus-mediated retinal microvascular dysfunction and this signalling axis could include the therapeutic potential for other diseases involving pathological neovascularization and or macular edema [126].

4.4.4.4 Fractalkine

Fractalkine (CX3CL1) is an intriguing chemokine that plays a central role in the nervous system. Expression of CX3CL1 on neurons and its receptor CX3CR1 on microglia facilitates a privileged interaction, playing important roles in regulating the function and maturation of these cells. CX3CL1 is reported to have neuroprotective and anti-inflammatory activities [127]. Studies suggest that dysregulated microglial activation via loss of FKN/CX3CR1 signalling disrupts the vascular integrity in retina during systemic inflammation [128].

4.4.4.5 Macrophage migration inhibitory factor

Macrophage migration inhibitory factor (MIF) is a chemokine that stimulates macrophages causing their recruitment at sites of inflammation, increasing their adherence, motility, and phagocytosis. It also prevents random migration of macrophages [129]. Studies indicate increased levels of MIF in the vitreous of patients with PDR and a significant association between MIF levels and grades of fibrous proliferation, suggesting the possibility that MIF may play a part in the development of the proliferative phase of PDR [130].

4.4.5 Intercellular adhesion molecule-1 (ICAM1)

ICAM-1 is a cell surface glycoprotein, which serves as an adhesion receptor that is known for regulating leukocyte recruitment from circulation to sites of inflammation. In addition to vascular endothelial cells, ICAM-1 expression is also induced on epithelial and immune cells in response to inflammatory stimulation. ICAM-1 also serves as a biosensor to transduce outside-in-signalling via association of its cytoplasmic domain with the actin cytoskeleton following ligand engagement of the extracellular domain. Thus, ICAM-1 has emerged as a master regulator of many essential cellular functions both at the onset and at the resolution of pathologic conditions [131]. Increased expression of adhesion molecule leads to activation of RAGE, oxidative stress, vascular leakage in the diabetic retina, capillary non perfusion, and damage of endothelial cells and the adhesion of leukocytes to the endothelium and expression of retinal vascular adhesion molecules such as VEGF [132]. ICAM-1 is the primary adhesion molecule involved in DR and its levels in vitreous are elevated in patients with active PDR [114].

5. Therapy aimed at inflammatory targets

Maintenance of a good glycemic control is considered to be the most important modifiable factor influencing the stage and progression of diabetic retinopathy. However, inhibition of retinal inflammation may also reduce the degree of

retinopathy despite the presence of a hyperglycaemic state. High-dose aspirin, COX-2 inhibitors and corticosteroids have been used, either experimentally in animal models or therapeutically in humans, and found to have a beneficial effect in reducing DR changes. However, these drugs are associated with unfavourable side effects especially when used over a long-term course. Hence other alternatives should be looked at. These alternatives can include RAGE inhibitors, minocycline, derivatives of salicylates and inhibitors of TNF-α and 5-lipoxygenase. Salicylates inhibit the nuclear migration and possibly activation of NF-κB in retinal neurons [133]. Evidence is accumulating showing that lipid supplementation with omega 3 polyunsaturated fatty acids (especially DHA) has a beneficial effect in DR [134].

Retinal inflammatory changes in diabetics have been found to be inhibited by therapies wherein the primary target is at a different site. The antihypertensive telmisartan, angiotensin receptor blocker (Type I receptor) was found to suppress retinal leukostasis and expression of VEGF and ICAM-1 [135]. Similarly, in diabetic animal models, Candesartan reduces the presence of acellular retinal capillaries, iNOS and nitric oxide [136]. The beneficial effect of statins on DR has also been reported by Kang et al. in 2019 [137]. The authors evaluated patients with diabetes and dyslipidemia and found that statin use was associated with a decreased prevalence of DR and a lower need for invasive therapy for vision threatening diabetic retinopathy complications. This therapeutic benefit can be attributed to the pleiotropic property of statins, which also function as anti-inflammatory agents. Tuuminen et al. [138] found a decreased intravitreal levels of pro-angiogenic factors, transforming growth factor B1 and matrix metalloproteinase 9 in individuals treated with simvastatin.

The role of salicylates in DR has been studied extensively, following the initial reports by Powell and Field in 1964 [20]. Administration of aspirin in animal models has found to reduce retinal capillary degeneration but conflicting results were reported in human trials. The Early Treatment Diabetic Retinopathy Study (ETDRS) is particularly of note in this case. The ETDRS report number 8 results indicated that aspirin has no clinically beneficial effect on the progression of retinopathy in individuals taking 650 mg of aspirin per day [139]. However, we have to take into account that the anti-inflammatory dose of aspirin is much higher than what was being administered in the study. Salicylates have shown to reduce insulin resistance in the retina in a Type II diabetic rat model as per Jiang Y and co-workers [140].

TNF-α is a key molecule in the inflammatory puzzle thus it serves as an attractive pharmacological target. Subcutaneous injection of TNF-α trap (Eternacept) was found to significantly reduce retinal inflammation, retinal cell injury and vascular permeability in diabetic rats [141]. However, no clinical trials have reported this effect till date. A small pilot study of 4 patients who were administered Infliximab (TNF-α antibody) showed a decrease in central macular thickness and a corresponding improvement in visual acuity [142].

Inhibition of leukostasis is another mechanism that can be targeted in anti-inflammatory therapy for DR. Leukocyte function associated antigen-1 (LFA-1) is an integrin molecule and is extremely important for leukocyte-endothelial cell interactions. SAR-1118 is a topical antagonist of LFA-1 and has shown a dose dependant reduction of leukostasis and vascular leakage in a diabetic rat model [143]. Anti CD49a neutralising antibody blocks the interaction between very late antigen-4 (VLA-4) and VCAM-1 and has also shows efficacy in reducing leukostasis [144].

Apart from their antibiotic activity both Minocycline and Doxycycline are known to possess neuro-protective and immunomodulatory properties, such as inhibiting production of NO, prostaglandins, TNF-α and caspases [145]. A small

study of minocycline in 5 patients with DME showed improvement in visual acuity with reduction in macular edema [146]. Another study involving doxycycline demonstrated an improvement in perimetric parameters in individuals with severe NPDR or PDR [147].

Photobiomodulation is another prospective therapy which has shown promise in a small clinical study of patient with non-centre involving diabetic macular edema [148]. It consists of series of brief illumination with specific wavelengths of light emitted from a laser. It has shown to affect the signalling pathways within the cells and inhibits diabetes induced leukostasis, ICAM-1 expression and production of reactive oxygen species [149].

6. Conclusion

Studies carried out both in diabetic patients and experimental animal models of diabetic retina have shown that the diabetic milieu promotes an increased local expression of inflammation. Unlike, uveitis however this inflammation is not clinically apparent and is noted at a molecular level. Critically located between the vasculature and neurons of the retina, Glial cells have a key role in closely regulating the retinal microenvironment. Recent findings implicate that these cells also responsible in the initiation of the inflammatory cascade.

It is possible that inflammation does not perfectly describe all the changes that ultimately occur in diabetic retinopathy, but it does seems to describe the pathogenesis of the retinopathy better than the previous concept of microvasculopathy. It is likely that this concept will become better focused with future research.

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