Role of inflammation in diabetic retinopathy: therapeutic targets

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Inflammation plays a key role in the pathogenesis of diabetic retinopathy (DR), leading to alterations in the blood–retinal barrier and increased vascular permeability. Many anti-VEGF medications are now available for the treatment of DR, but response to these medications is not as robust in patients with diabetic macular oedema. Newer biologic agents are currently under study to improve the treatment of DR. These have shown promising results to both decrease the treatment burden of intravitreal injections and improve visual outcomes for diabetic patients.

Keywords: Chemokines, cytokines, diabetic retinopathy, drug therapy, inflammation.

Introduction

DIABETES mellitus is a global epidemic, currently affecting over 415 million adults. This number is projected to rise to 642 million by the year 2040 (ref. 1). Diabetic retinopathy (DR) is a vision-threatening, microvascular complication of long-standing diabetes that is present in approximately 35% of diabetics[2] and represents the leading cause of blindness in the 20- to 64-year-old population in developed countries[3]. Individuals with diabetes typically go through phases of no retinopathy followed by mild to moderate non-proliferative retinopathy. Over a 14-year period, more than half of the individuals with DR go on to develop vision-threatening diseases, including diabetic macular oedema (DME), proliferative diabetic retinopathy (PDR), and/or macular ischaemia[4].

Until recently, photocoagulation (along with modification of risk factors) has been the only treatment for vision-threatening DR, applied focally for DME[5] and panretinally for PDR[6]. Although beneficial, these treatments are associated with a number of complications, including pain, loss of peripheral and night vision, intraretinal fluid accumulation, vitreous hemorrhage and, rarely, retinal detachment. In the past decade, anti-vascular endothelial growth factor (VEGF) drugs have been shown to significantly improve the neovascularization associated with PDR. Though they can also improve visual outcomes in DME, their effect in the latter is not as robust[7]. Even more recently, other inflammatory mediators have been identified that contribute to the pathogenesis of DR, and many of these are now being targeted by various novel drugs.

In this review, we will briefly discuss the pathophysiology of DR, focusing on alterations of the blood–retinal barrier (BRB) and the role of inflammation. We will then explore the various medical treatments used in DR, including anti-VEGF drugs, steroids, non-steroidal anti-inflammatory drugs (NSAIDs), and conclude with new biologics that are currently under study.

Blood–retinal barrier

Similar to the blood–brain barrier, the BRB is a tight, physiologic barrier that regulates ion, protein and water movement into and out of the retina, and is composed of both an outer and an inner portion. The inner BRB is formed by tight junctions between retinal capillary endothelial cells, and its integrity is maintained by adhesive interactions between endothelial cells and associated pericytes[8,9]. The outer BRB consists of the retinal pigment epithelium cells, located between the fenestrated choriocapillaris and the outer retina[10]. The inner BRB nourishes the inner retina, removes toxins and provides a barrier between the retina and the blood, whereas the outer BRB maintains homeostasis of the outer retina[11]. An intact BRB is essential in maintaining normal visual function through these processes.

Diabetes leads to three essential changes in the BRB: breakdown of tight junctions between the endothelial cells, pericyte loss and basement membrane thickening[10]. Loss of cell-to-cell junctions in the endothelium results in the leakage of lipids, plasma and red blood cells. This leakage can be seen clinically on funduscopic exam as hard exudates, oedema and intraretinal haemorrhage respectively. Pericytes are contractile cells that regulate blood flow in the microcirculation[12], and their loss leads to endothelial cell proliferation and microaneurysms, the

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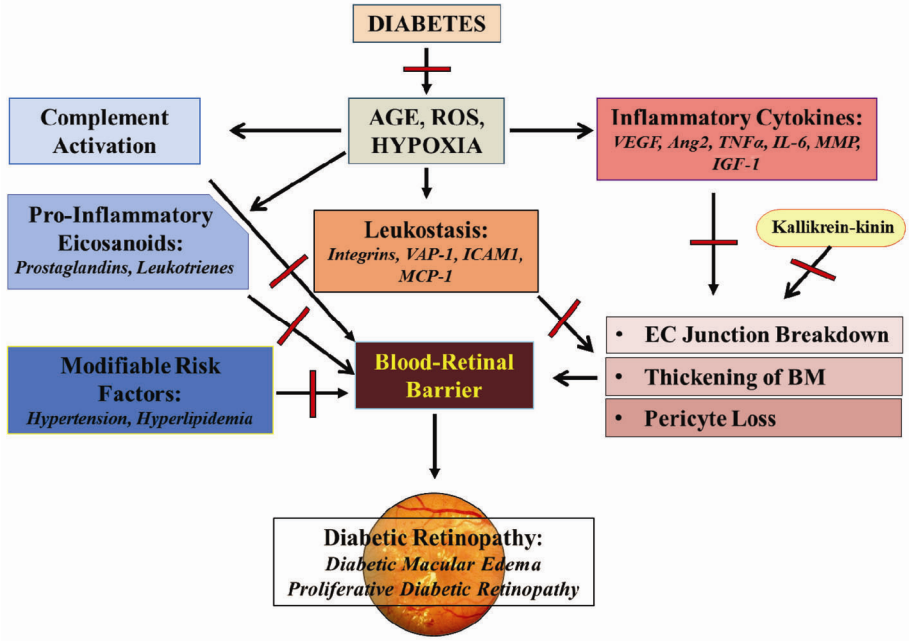

Figure 1. Flow diagram outlining factors that contribute to alterations in the blood–retinal barrier, leading to the development of diabetic retinopathy (DR). Current targets of interest aimed at preventing and treating DR are shown as red rectangles. AGE, Advanced glycation end-products; ROS, Reactive oxygen species; VEGF, Vascular endothelial growth factor; Ang-2, Angiopoietin 2; TNFa, Tumour necrosis factor alpha; IL-6, Interleukin 6; MMP, Matrix metalloproteinase; IGF-1, Insulin-like growth factor 1; VAP-1, Vascular adhesion protein 1; ICAM-1, Intercellular adhesion molecule 1; MCP-1 (monocyte chemoattractant protein-1), Chemokine ligand 2 and BM, Basement membrane.

earliest clinical lesions present in diabetic retinopathy[13]. It is unclear how basement membrane thickening contributes to the pathogenesis of DR, but it may be related to an alteration in the molecular structure or distribution of negatively charged proteoglycan molecules that leads to increased porosity[14]. The upregulation of cytokines and other inflammatory mediators likely contributes to these three important changes characteristic of the BRB in DR[15], involving four major biochemical pathways (polyol pathway, advanced glycation end-product pathway, protein kinase C pathway and hexosamine pathway), all leading to increased oxidative stress and inflammation[16]. Each of these pathways represents a potential target for novel therapies in the treatment of DR (Figure 1).

Inflammation and breakdown of the blood–retinal barrier

Chronic hyperglycaemia induces multiple cellular changes through direct toxic effects of glucose, including oxidation, hyperosmolarity and the formation of advanced glycation end-products. Incubation of retinal cells in a glucose-rich environment promotes the upregulation of inducible nitric oxide synthase (iNOS), cyclooxygenase 2 (COX-2), and 5-lipoxygenase, all of which are pro-inflammatory[17]. It is clear that hyperglycaemia and the products of glucose metabolism create sustained changes in cell signalling pathways, leading to the activation of protein kinase C and the upregulation of multiple inflammatory mediators[15,17,18].

Leukocyte recruitment is the hallmark of inflammation, including that seen in DR. In the lumen of retinal blood vessels, leukocytes continuously roll along the endothelium. They begin to express integrins due to the release of chemokines from activated endothelial cells from chronic inflammation induced by hyperglycaemia, allowing monocytes, neutrophils and other leukocytes to bind ligands expressed by the activated endothelial cells. The integrin-ligand interactions lead to stable adhesion (leukostasis). These cells then undergo migration through the endothelium and pierce through the basement membrane (diapedesis), where they finally become activated macrophages and begin to produces various cytokines, perpetuating the inflammatory response and activating local microglia (Figure 2)[19].

Cellular changes from inflammation in diabetic retinopathy

Leukocyte adhesion plays a central role in retinal endothelial cell injury and death, leading to breakdown of the BRB[20]. Increased intravascular neutrophils are present in areas of capillary non-perfusion in the retinas of diabetic monkeys; similarly, elevated leukocytes are present in choroidal vessels of diabetic humans[21,22]. Intercellular adhesion molecule-1 (ICAM-1), an integrin ligand known

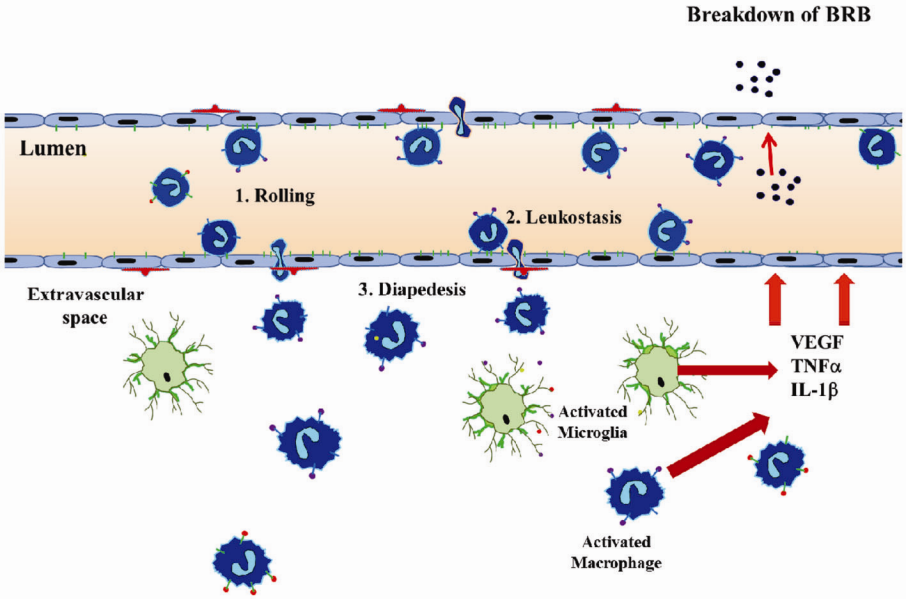

Figure 2. Diagram demonstrating leukocyte recruitment in the retinal vasculature. Monocytes are continuously rolling along the endothelium. When activated, the endothelium begins to express integrin ligands, leading to leukostasis and allowing the monocytes to undergo diapedesis, where they become macrophages, activate microglia and produce inflammatory cytokines. IL-1β, Interleukin 1 beta.

to play a key role in leukostasis, is known to be upregulated on retinal endothelial cells in diabetes, and inhibition of ICAM-1 may prevent vascular leakage in diabetic retinopathy[23].

Inflammatory changes may also be partially responsible for pericytes dropout, one of the key changes responsible for disruption of the BRB in DR. Retinal pericytes incubated in high glucose upregulate numerous inflammatory mediators, including interleukin-1β (IL-1β), nuclear factor kappa b (NF-κB), VEGF, tumour necrosis factor alpha (TNF-α), transforming growth factor beta (TGF-β) and ICAM-1. Elevation of these inflammatory mediators leads to pericyte cell death, and the inflammation continues even after normalization of glucose[24].

Müller cells, which play an essential supportive roll in the retina, also respond to the inflammatory changes in DR. They are a major source of retinal VEGF, which promotes retinal inflammation, neovascularization and oedema seen in DR[25]. Inhibition of Müller cell-derived VEGF in diabetic mice significantly decreases the expression of multiple inflammatory mediators, including TNF-α, ICAM-1 and NF-κB (ref. 26).

Microglia are native phagocytic cells that play a principal role in regulating inflammation. When activated, they release pro-inflammatory and neurotoxic substances[17]. As inflammatory cytokines increase in the presence of prolonged hyperglycaemia, microglia become activated, increase in number and disseminate throughout the retina[27]. Hyperglycaemia may independently activate microglia through interactions with glycated compounds, leading to the release of TNF-α (ref. 28). Microglia act to perpetuate the inflammatory response in DR, leading to chronic inflammation and apoptosis of various cells. Variations in microglial genetics may account for differences in a patient's susceptibility to DR[27].

Inflammatory mediators in diabetic retinopathy

There are many players involved in the inflammatory response seen in DR (Figure 3); VEGF is one of them. It is a pro-inflammatory cytokine that is greatly implicated in the pathogenesis of DR. It has been the subject of intense research for more than a decade, being consistently elevated in the vitreous of patients with DR[29]. There also appears to be a dose–response relationship between VEGF and DR, with higher vitreous levels of VEGF corresponding with worse disease[30]. VEGF is upregulated by hypoxia (via hypoxia-inducible factor 1), hyperglycaemia (via advanced glycation end-products and pro-inflammatory cytokines), and a variety of growth factors[31], all of which are present in DR. VEGF is synthesized by numerous cells, including retinal pigmented epithelial cells, pericytes, endothelial cells, Müller cells and ganglion cells[32]. In addition to promoting angiogenesis and leading to neovascularization, it induces breakdown BRB and increases vascular leakage through activation of protein kinase C and disassembly of tight junctions[31,33]. VEGF is now recognized as a key pro-inflammatory cytokine in DR that promotes ICAM-1 expression (causing increased leukostasis), induces neovascularization and increases vascular permeability[15].

In addition to VEGF, a number of cytokines and chemokines are elevated in the vitreous of patients with DR. One of these, TNF-α, plays an important role in diabetes-induced degeneration of retinal capillaries. Soluble TNF-α inhibitors have been shown to reduce leukocyte adherence in retinal blood vessels, decrease BRB

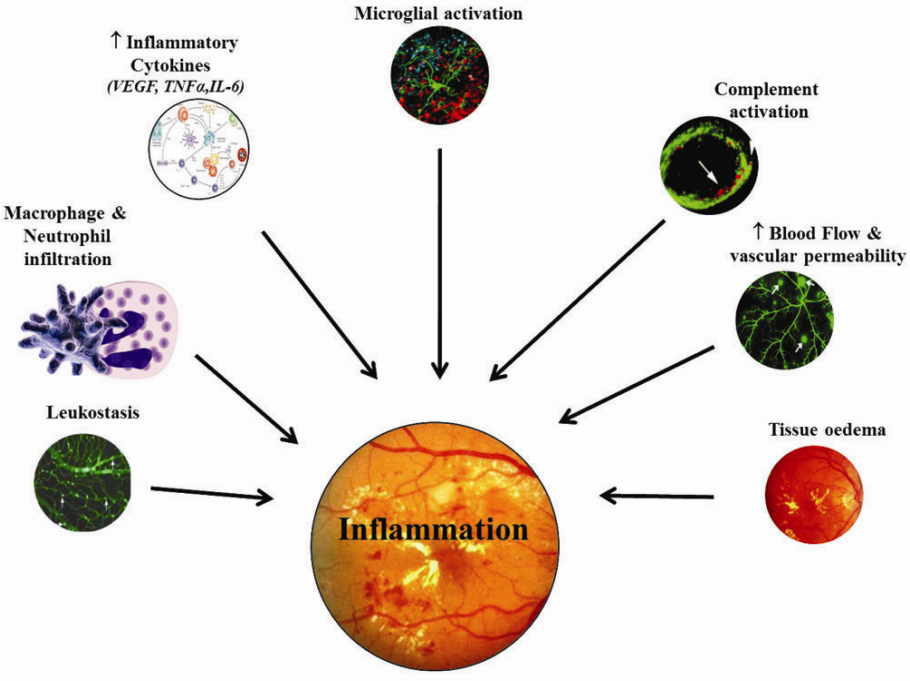

Figure 3. Contributors of inflammation present in the eyes with DR.

breakdown, and prevent pericyte loss and capillary degeneration[17]. Another cytokine, IL-1β, also contributes to the degeneration of retinal capillaries. Diabetic mice lacking IL-1β are protected from retinal capillary degeneration[34]. Chemokine ligand 2 (CCL2), also known as monocyte chemotactic protein-1 (MCP-1), is another mediator that may contribute to early immune cell recruitment, and CCL2 knockout mice show significant reductions in vascular permeability and monocyte infiltration after streptozocin-induction of diabetes[35].

Angiopoietin 2 (Ang-2) is a cytokine involved in controlling microvascular permeability and cell death. It has been shown to be upregulated by hyperglycaemia in the retina of diabetic animals. Increased Ang-2 leads to vascular permeability by altering the function of vascular endothelial cadherin, which plays an important role in maintaining a tight endothelial barrier. Ang-2 upregulation likely plays an important role in the vasopermeability seen in DR[9].

ICAM-1 plays a role in the adhesion of leukocytes to the endothelial cell wall. It is upregulated by VEGF, oxidative stress and NF-κB (ref. 17), all of which are increased in the retinas of diabetic patients. Elevation of ICAM-1 is present in diabetic rats, accompanied by increased leukostasis and vascular permeability. These changes are significantly reduced after administration of an anti-ICAM-1 antibody. Interestingly, inhibiting ICAM-1 also leads to reduced endothelial cell apoptosis, implying that ICAM-1 may also play a role in regulating Fas–Fas ligand-mediated apoptotic events by increasing the adherence of leukocytes to the retinal vasculature[15].

The complement system plays an important role in inflammatory and immune responses, and its abnormalities lead to many common autoimmune and inflammatory disorders. Complement activation has been shown to play an important role in DR. The terminal product of complement activation, the C5b-9 membrane attack complex, and C3d have been identified in the choriocapillaris of eyes with DR, as well as within the walls of small and midsized retinal vessels[15]. Elevated levels of complement factor B and C4 have been seen in more advanced disease, suggesting involvement of both the alternative and classical pathways of the complement system[36].

Diabetes has also been shown to induce changes in retinal fatty acid metabolism, leading to increased proinflammatory eicosanoids, including both prostaglandins (via the cyclooxygenase pathway) and leukotrienes (via the lipoxygenase pathway)[17,37]. This may explain the strong association between dyslipidemia and the development of DR seen in the diabetes control and complications trial/epidemiology of diabetes interventions and complications study (DCCT/EDIC)[38]. The hyperosmolarity related to hyperglycaemia appears to activate COX-2 and promote inflammation through prostaglandin E2, leading to worsened retinopathy in diabetic patients; this effect is significantly reduced by selectively inhibiting COX-2, which in turn reduces VEGF mRNA[39,40]. The leukotrienes, products of 5-lipoxygenase, play an important role in leukocyte recruitment and vascular permeability, and they are increased in the vitreous of diabetic patients[17,41]. Recently, a study showed that increased intake of long-chain ω-3 polyunsaturated fatty acids can

decrease the progression of DR[42], possibly by modulating the amount and type of eicosanoids made[43].

Targeting inflammation to treat diabetic retinopathy

The most important treatment for DR is to prevent its development and progression through controlling its risk factors. The DCCT and the United Kingdom Prospective Study (UKPDS) showed that tighter glycaemic control was associated with a decreased risk of retinopathy. The UKPDS also showed that intensive blood pressure management can reduce the progression of retinopathy[44,45]. In 1998, the Early Treatment Diabetic Retinopathy Study (ETDRS) published data supporting these findings, and suggested that reducing blood lipids and treating anaemia might also slowdown the progression of retinopathy[46].

The ETDRS published its first report in 1985, revealing that focal photocoagulation of DME led to substantial reduction in visual loss[5]. Focal/grid laser continues to be a mainstay in the treatment of DME, where it reduces intraretinal fluid and decreases inflammation. Pan-retinal photocoagulation, on the other hand, is used in PDR, where the goal is to create scars on the peripheral retina, leading to decreased oxygen demand and subsequently halting the production of VEGF. More recently, numerous drugs have been developed that directly inhibit VEGF, each with very promising outcomes in treating both PDR and DME. Encouraged by these results, other inflammatory mediators are now being targeted in the hope of improving visual outcomes for patients with DR.

Anti-VEGF medications

There are several intravitreal anti-VEGF medications currently used to treat DR. These medications tend to be used for centre-involving DME, whereas focal/grid laser is typically employed in non-centre-involving disease. They can also be employed in the treatment of PDR, either alone or as an adjunct to laser treatment, demonstrating similar efficacy as pan-retinal photocoagulation in reducing the risk of intraocular bleeding[47–50]. However, it is still unknown if the beneficial effects of anti-VEGF medications will continue after cessation of intravitreal injections.

Molecules that directly inhibit VEGF include the anti-VEGF aptamer pegaptanib, the monoclonal antibody fragment ranibizumab and the full-length antibody bevacizumab. Although not approved for treating DR, bevacizumab is a popular 'off-label' drug due to its lower cost and demonstrated effectiveness[10]. Other anti-VEGF medications include soluble VEGF receptor analogs such as VEGF-Trap and small interfering RNAs such as bevasiranib and rapamycin. Aflibercept is a newer medication that is a soluble VEGF receptor with approximately 100 times greater affinity to VEGF-A than either bevacizumab or ranibizumab, leading to a longer half-life and longer intervals between injections[10].

The most commonly used anti-VEGF medications in practice are bevacizumab, ranibizumab and aflibercept. All three of these agents have shown efficacy in improving visual acuity in patients with centre-involving DME. When compared head-to-head with ranibizumab and bevacizumab, aflibercept showed greater visual acuity improvement over one year in patients with worse overall disease (visual acuity of 20/50 or worse). However, when visual acuity is relatively good (20/40 or better), the cost-effectiveness of bevacizumab likely outweighs the potential visual acuity gains provided by either aflibercept or ranibizumab[51]. Although anti-VEGF medications have vastly improved the treatment of DR, there are many patients with DME who do not respond to treatment, suggesting other key inflammatory factors that may play an even more important role than VEGF in promoting vascular permeability[7,10].

It is also possible that DME patients who do not respond to anti-VEGF medications may need much higher doses due to higher levels of VEGF. However, the Ranibizumab for Edema of the mAcula in Diabetes–Protocol 3 (READ-3) study showed greater efficacy with lower dose treatment in DME patients at 24-month follow-up[52]. Similar outcomes were seen in the RISE and RIDE trials, phase-3 trials comparing monthly ranibizumab to sham injections, at 36-month follow-up[53]. To prevent potential side effects with apparently equivalent outcomes, the lower dose of ranibizumab (0.3 mg) is now approved for treating DME.

Designed ankyrin repeat proteins (DARPins) are novel molecules that are able to target VEGF-A with a higher potency and longer half-life[54]. One such molecule, abicipar, is currently under investigation for the treatment of DME. Phase-2 clinical trials were completed in July 2015 and phase-3 trials are currently in progress; study results have not yet been posted[55]. Other novel therapies are attempting to target multiple growth factors. One such molecule is squalamine, which targets VEGF, platelet-derived growth factor and basic fibroblast growth factor. Phase-2 clinical trials completed in 2015 showed better visual improvement using a combination of squalamine eye dross and ranibizumab in comparison to ranibizumab alone in the treatment of choroidal neovascularization, retinal vein occlusion and wet ARMD (age related macular degeneration)[56]. Notwithstanding these promising results, a study with squalamine eye drops in patients with DME was withdrawn prior to enrollment in 2015 (ref. 57).

Novel delivery systems of anti-VEGF medications may also prove to reduce the treatment burden for patients with DR. These include bioerodible implants and microspheres, encapsulated cells and gene therapy. One such delivery system, a refillable non-biodegradable implant, is currently being studied for wet ARMD. If the results

are promising, this may be an effective treatment option for DR[58]. Another phase-2 study in wet ARMD patients that may be applicable to DR is using NT-503 encapsulated cell therapy, where an implantable retinal pigmented epithelium cell line produces a soluble VEGF receptor protein. Again, no study results have been posted as of now[59].

Steroids

Glucocorticoids are crucial in the treatment of many inflammatory disorders, as they have broad anti-inflammatory effects. These effects are mediated both by direct binding of the steroid/steroid receptor complex in the promoter region of genes, or by interacting with other transcription factors (especially NF-κB). They act to inhibit a number of cytokines, chemokines, inflammatory eicosanoids and adhesion molecules that play a role in both acute and chronic inflammation[60]. Diabetic patients have significantly higher concentrations of IL-1β, IL-6, IL-8, CCL2 and VEGF in the aqueous humor[61]. In contrast to anti-VEGF medications, which have been shown only to significantly reduce the aqueous levels of VEGF, intravitreal steroids (triamcinolone acetonide) have been shown to significantly decrease IL-6, interferon-induced protein-10, CCL2, platelet-derived growth factor-AA and VEGF in DME patients[62].

Triamcinolone is as effective in reducing central retinal thickness in patients with DME as ranibizumab, although the effectiveness gradually diminishes due to increased rates of cataract formation. However, triamcinolone plus laser in pseudophakic DME patients was found to be superior to laser alone and equivalent to ranibizumab; it may be an effective treatment in anti-VEGF non-responders[10,63]. In addition to triamcinolone injections, dexamethasone intravitreal implants and fluocinolone acetonide intravitreal inserts have shown substantial benefit in improving visual acuity in DME patients[64,65]. However, all of these treatments carry the significant risk of increased intraocular pressure and cataract formation and are typically reserved for patients who respond poorly to anti-VEGF therapy[10].

Non-steroidal anti-inflammatory drugs

NSAIDs are among the most commonly used medications in the world. Since they do not exhibit the same effects as steroids, they do not pose the same risks of increased intraocular pressure or cataract formation. They function through the inhibition of cyclooxygenase enzymes and the formation of prostaglandins. They are commonly used in the treatment of cystoid macular oedema, and to control and prevent the inflammation induced by cataract surgery[10].

Although NSAIDs may seem like a promising strategy to treat and prevent DR, animal models showed the dose of aspirin needed to prevent the BRB breakdown may lead to severe side effects in humans[66]. An early study showed that aspirin with or without dipyridamole slowed the progression of microaneurysm evolution in early DR[67]. However, the ETDRS study showed that aspirin did not decrease the risk of visual loss in DME or high-risk PDR (nor did it increase the risk of vitreous haemorrhage)[68]. A recent phase-2 study (DRCR Protocol R) showed that administration of NSAID nepafenac 0.1% eye drops three times daily for a year does not significantly improve visual acuity or decrease central retinal thickness in patients with noncentral-involved DME[69].

Cytokine inhibitors

Ang-2, which has been shown to destabilize blood vessels, binds to the endothelial receptor tyrosine kinase Tie-2. A phase-2 clinical trial recently showed that at 12 weeks, subcutaneous administration of the Tie2 activator AKB-9778 plus intravitreal ranibizumab caused a significantly greater reduction in DME than that seen in with ranibizumab monotherapy[70]. Subcutaneous administration of the medication is one advantage of this novel therapy. Another phase-2 trial (BOULEVARD) is currently enrolling patients with centre-involving DME to study the efficacy of an intravitreal bispecific antibody with anti-VEGF and anti-Ang-2 properties. Patients will be treated and followed for a total of 36 weeks[71].

Another important inflammatory cytokine is TNF-α. Infliximab is an anti-TNF-α monoclonal antibody commonly used to treat inflammatory disorders such as ankylosing spondylitis, rheumatoid arthritis and Crohn's disease. A relatively small phase-3 trial of patients with DME showed improved visual acuity with IV administration of infliximab plus laser photocoagulation compared with photocoagulation alone[72]. Although these results are promising, larger trials are needed to prove the efficacy of these drugs in DR.

IL-6 is another inflammatory cytokine consistently shown to be elevated in the aqueous humor of diabetic patients[61]. Systemic IL-6 blockers have been shown to decrease VEGF levels in rheumatoid arthritis and cystoid macular oedema in patients with uveitis. A clinical trial using an intravitreal IL-6 antibody is currently under way, and the drug appears to be well tolerated in animal models with a longer half-life than either aflibercept or ranibizumab[73].

Other novel inhibitors

Let us now consider other novel inhibitors[74]. CCL2, a chemokine that plays a critical role in inflammation by promoting retinal monocyte trafficking and activating

microglia, is significantly elevated in early DR, even more so than VEGF or IL-6 (ref. 75). An oral inhibitor targeting the CCR2/CCR5 receptor was shown to significantly reduce urine albumin to creatinine ratio at 12 weeks in patients with overt DR[76]. A phase-2 trial was under way to compare the efficacy of the same oral CCR2/5 inhibitor in the treatment of DME, but it was terminated early in October 2015 for undisclosed reasons; no results have been reported[77].

The kallikrein–kinin system is another contributor to DME. Activation of this system has been shown to cause retinal thickening and increased retinal vascular permeability, and increased prekallikrein and kallikrein levels are elevated in the vitreous of patients with DME. These changes appear to be related to oxidative stress (iNOS) and are independent of VEGF[78]. A phase-1 study of an intravitreal plasma kallikrein inhibitor recently demonstrated its safety, tolerability and pharmacodynamics in patients with centre-involving DME[79]. Phase-2 trials are now under way.

Integrins are involved in the early stages of inflammation, leukostasis and adherence of leukocytes to the endothelium. They are present on leukocytes and bind activated ligands (such as ICAM-1) present on the endothelium of blood vessels[19]. A phase-2 trial of an integrin peptide is currently under way. To date, this novel drug has been shown to reduce vascular leakage and inhibit new blood vessel formation in DR[80]. It may prove to be another promising treatment to reduce the burden of intravitreal injections.

Conclusion

Inflammation and BRB breakdown are key changes present in DR. This understanding has led to the development of many new treatments aimed at preventing and reversing the inflammatory changes in diabetic retinas. In the last decade, anti-VEGF agents have transformed our treatment of DR and have been proven to be no less effective than panretinal photocoagulation in PDR patients. However, not all DME patients respond as well to anti-VEGF medications. This is likely due to numerous inflammatory pathways that are activated independent of VEGF. Many cytokines and chemokines are currently under investigation that may play a more important role in BRB breakdown, leading to a better understanding of potential targets in the treatment of DR, a leading cause of blindness worldwide.

Conflicts of interest: There are no conflicts of interest to disclose.

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