

Role of Cytokines and Trophic Factors in the Pathogenesis of Diabetic Retinopathy

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Abstract: Diabetic retinopathy (DR) is one of the most frequent complications of diabetes and the leading cause of acquired blindness in developed countries. A note worthy problem in DR is the formation of fibrovascular epiretinal membranes (ERMs) which can cause tractional retinal detachment in the progressed stage of DR. Ocular vitreous fluid and ERMs, which can be obtained during vitrectomy, allow laboratory studies investigating the pathogenesis of DR. Recent studies have shown a significant association between clinical grades of DR and the expression levels of specific cytokines, such as vascular endothelial growth factor (VEGF), in the intraocular fluid. In addition, expression of various trophic factors and their receptors are reported in ERMs. ERM is composed of many cell types including endothelial cells, which is the primary target of glucose-induced dysfunction in the retina. However, some trophic factor receptors are observed in other cell types such as the glial cells, and their role in ERMs is unclarified. These findings may uncover the detailed pathogenesis of DR, which may lead to new therapeutic strategies. This review briefly summarizes recent research regarding the clinical and laboratory findings of DR.

Keywords: Diabetic retinopathy, epiretinal membrane, cytokine, trophic factor, vascular endothelial cell, glia.

INTRODUCTION

Diabetic retinopathy (DR) is one of the most common complications in diabetes, and affects nearly all patients with type 1 diabetes and more than 60% of type 2 diabetes within 15 years after diagnosis [1-5]. The risk of blindness is about 25 times greater in people with diabetes than non-diabetics [6]. Hyperglycemia is known to be the primary pathogenic factor, but the mechanisms by which elevated blood glucose causes retinal damage are not yet clear. DR is a progressive disease affecting the structure and cellular composition of the microvasculature [7-9]. DR can be divided into three clinical stages: simple retinopathy, preproliferative retinopathy, and proliferative retinopathy [10-13]. Simple retinopathy is the earliest stage and characterized by capillary wall thickening, pericyte loss, increased leukocyte adhesion to the vessel wall, and alternations in blood flow [14]. As the disease progresses, 'dot' and 'blot' hemorrhages and hard exudate are observed by ophthalmoscope examination (Fig. 1A,B). Since retinal vessels become increasingly damaged from diabetes, progressive endothelial cell loss leads to the occlusion of retinal capillaries and formation of non-perfusion (ischemic) areas. Preproliferative DR develops in some eyes that initially show only simple DR (Fig. 1C). In this stage, intraretinal microvascular abnormalities (IRMA) and soft exudates are frequently observed adjacent to area of capillary closure. Proliferative diabetic retinopathy (PDR) develops following the

enlargement of the ischemic region (Fig. 1D,E). Retinal ischemia induces angiogenic factors, such as vascular endothelial growth factor (VEGF), in order to make up for oxygen deficiency [15,16]. However, uncontrolled upregulation of these angiogenic factors in DR results in pathologic angiogenesis (neovascularization). Since the walls of these new vessels are fragile and easy to break, untreated neovascularization can lead to PDR, which causes severe bleeding of the vitreous cavity. Furthermore, progressive contraction of fibrovascular epiretinal membranes (ERMs) over large areas of vitreoretinal adhesion can cause tractional retinal detachment (Fig. 1F) which results in blindness. It has been estimated that without treatment, 50% of all PDR patients will become blind within five years following diagnosis [17]. To prevent the enlargement of avascular areas, neovascularization, and recurrent vitreous bleeding, laser photocoagulation is the current main treatment for DR. The procedure consists of burning the retina with a laser beam. The laser beam energy is absorbed by pigmented cells, such as retinal pigment epithelial cells and choroidal melanocytes. The heat generated by the laser beam is transmitted to neighbouring cells, thus causing photoreceptor coagulation. The mechanism by which laser photocoagulation induces regression of neovascularization is controversial, but it is likely that the source of the growth factor is destroyed and the decrease in growth factor concentration suppresses neovascularization [14]. Furthermore, photocoagulation of retinal pigment epithelial cells in vitro induces the synthesis of a vascular endothelial cell proliferation inhibitor, most probably transforming growth factor- (TGF-) [18]. Laser photocoagulation at the initial stage of PDR reduces the incidence of severe vision loss from 50% to approximately 5% over 5 years [19].

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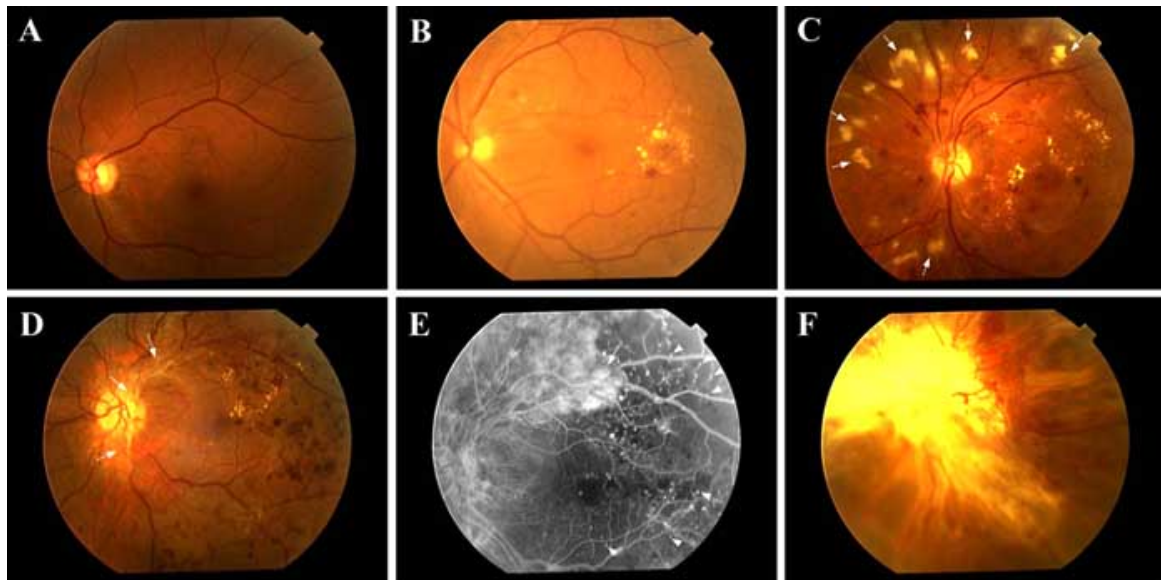


Fig. (1). **A**, Normal fundus without diabetic retinopathy (a 74-year-old woman). **B**, Fundus photograph of simple diabetic retinopathy (a 55-year-old man). Retinal hemorrhage and hard exudates are observed. **C**, Fundus photograph of preproliferative diabetic retinopathy (a 73-year-old man). Soft exudates (arrows) in addition to retinal hemorrhage and hard exudates are distributed in the posterior pole of the fundus. **D**, Fundus photograph of proliferative diabetic retinopathy (a 43-year-old man). Proliferative epiretinal membrane (arrows) is distributed along the arcade vessel and optic disc. **E**, Fluorescein angiography of proliferative diabetic retinopathy, corresponding to Figure 1D. A leakage from the proliferative epiretinal membrane (arrow), avascular area, and microaneurysm (arrowheads) are observed. **F**, Fundus photograph of proliferative diabetic retinopathy with retinal detachment (the same patient as Figure 1D and 1E). This patient refused surgery, therefore he developed total retinal detachment 8 months later and finally became blind.

For the laboratory studies investigating the pathogenesis of DR, ocular vitreous fluid, which is obtained during vitrectomy, is available. Since the vitreous is highly inert and protected by the blood-retina barrier, concentration of cytokines and growth factors in vitreous fluid is believed to reflect the production of these factors in human eyes. Several cytokines and growth factors are known to increase extracellular matrix protein deposition that is involved in angiogenesis [20]. We have examined the protein concentration of cytokines, such as macrophage migration inhibitory factor (MIF) and vascular endothelial growth factor (VEGF), and extracellular matrix proteins such as tenascin-C, in the vitreous samples [21-27]. Interestingly, there is a significant association between clinical grades of DR and vitreous levels of these cytokines or extracellular matrix. Fibrovascular ERMs, which are often observed in the PDR stage, is another available example. The prevalence of ERMs is 23% in 995 subjects with type 1 diabetes and 6% in 5411 subjects with type 2 diabetes [1,2]. We recently examined the expression of various trophic factor receptors in ERMs secondary to DR, and found high expression levels of GFR 2, one of the specific receptors for glial cell line-derived neurotrophic factor (GDNF), in the glial component [28]. Although the primary target of glucose-induced dysfunction is endothelial cells, glial cells might be another important cell type in understanding the pathogenesis of secondary ERMs. This review briefly summarizes recent research regarding the clinical and laboratory findings of DR.

Possible Involvement of Cytokines and Trophic Factors in the Pathogenesis of Diabetic Retinopathy

Table 1 is a summary of representative studies describing the involvement of cytokines and trophic factors in DR. We will discuss the role of these specific factors in accordance with our recent research results.

Monocyte Chemotactic Protein-1 (MCP-1)

As summarized in the introduction, retinal ischemia due to capillary occlusion plays a crucial role in the development of DR. One of the possible mechanisms of capillary occlusion is an increased macrophage adhesion to the endothelium. Experimental studies have shown an association between macrophage activation and retinal angiogenesis [29,30]. In addition, immunohistochemical analysis determined the presence of intraocular macrophage in the human proliferative ERMs secondary to DR [31]. Since activated macrophages may produce multiple growth factors, which are involved in intraocular proliferation, these clinical and experimental studies suggest an important role of macrophages in the pathogenesis of DR. However, the precise mechanisms of how these immunocompetent cells recruit into the eyes are poorly understood.

Chemokines constitute a large family of structurally related small cytokines originally identified as factors regulating the migration of leukocytes in inflammatory and immune responses [32,33]. Recent studies have shown that MCP-1, which belongs to the CC subfamily of chemokines,

Table 1. Expression of cytokines, growth factors and their receptors in the eye of patients with diabetic retinopathy.

	Sample	Methods	Reference
MCP-1	Aqueous humor (n=49)	ELISA	Tashimo <i>et al.</i> , in press (37)
	Vitreous (n=30)	ELISA	Elnor <i>et al.</i> , 1995 (34)
	Vitreous (n=42)	ELISA	Abu El-Asrar <i>et al.</i> , 1997 (36)
	Vitreous (n=15)	ELISA	Capeans <i>et al.</i> , 1998 (35)
	Vitreous (n=52)	ELISA	Mitamura <i>et al.</i> , 2001 (24)
MIF	Aqueous humor (n=49)	ELISA	Tashimo <i>et al.</i> , in press (37)
	Vitreous (n=32)	ELISA	Mitamura <i>et al.</i> , 2000 (22)
VEGF	Aqueous humor (n=59)	Radioimmuno-assay	Aiello <i>et al.</i> , 1994 (59)
	Aqueous humor (n=35)	ELISA	Shinoda <i>et al.</i> , 1999 (48)
	Vitreous (n=8)	Time-resolved immunofluorometric assay	Adamis <i>et al.</i> , 1994 (58)
	Vitreous (n=76)	Radioimmuno-assay	Aiello <i>et al.</i> , 1994 (59)
	Vitreous (n=73)	ELISA	Katsura <i>et al.</i> , 1998 (47)
	Vitreous (n=31)	ELISA	Mitamura <i>et al.</i> , 2002 (25)
	Vitreous (n=27)	ELISA	Umeda <i>et al.</i> , 2002 (60)
	Vitreous (n=44)	ELISA	Funatsu <i>et al.</i> , 2003 (62)
	Epiretinal membrane (n=11)	immunohistochemistry	Chen <i>et al.</i> , 1997 (63)
	Epiretinal membrane (n=40)	ELISA	Armstrong <i>et al.</i> , 1998 (64)
PIGF	Epiretinal membrane (n=22)	RT-PCR/immunohistochemistry/ISH	Ishida <i>et al.</i> , 2000 (65)
	Retina (n=8)	RT-PCR	Spirin <i>et al.</i> , 1999 (42)
	Vitreous (n=4)	ELISA	Khaliq <i>et al.</i> , 1998 (67)
	Vitreous (n=31)	ELISA	Mitamura <i>et al.</i> , 2002 (25)
Tenascin	Epiretinal membrane (n=12)	immunohistochemistry	Khaliq <i>et al.</i> , 1998 (67)
	Retina (n=8)	RT-PCR	Spirin <i>et al.</i> , 1999 (42)
	Vitreous (n=58)	ELISA	Mitamura <i>et al.</i> , 2002 (26)
IGF	Epiretinal membrane (n=3)	immunohistochemistry	Immonen <i>et al.</i> , 1991 (51)
	Epiretinal membrane (n=8)	immunohistochemistry	Hagedorn <i>et al.</i> , 1993 (70)
	Retina (n=8)	RT-PCR / Northern blotting	Spirin <i>et al.</i> , 1999 (42)
	Vitreous (n=23)	Radioimmuno-assay	Grant <i>et al.</i> , 1986 (76)
bFGF	Vitreous (n=25)	Radioimmuno-assay	M-Scwickrath <i>et al.</i> , 1993 (77)
	Vitreous (n=51)	Radioimmuno-assay	Boulton <i>et al.</i> , 1997 (78)
	Vitreous (n=21)	Radioimmuno-assay	Burgos <i>et al.</i> , 2000 (71)
	Vitreous (n=8)	ELISA	Sivalingam <i>et al.</i> , 1990 (92)
HGF	Vitreous (n=51)	ELISA	Boulton <i>et al.</i> , 1997 (78)
	Epiretinal membrane (n=12)	Immunohistochemistry / ISH	Hueber <i>et al.</i> , 1996 (93)
	Epiretinal membrane (n=8)	Immunohistochemistry	Chen <i>et al.</i> , 1997 (63)
	Epiretinal membrane (n=30)	RT-PCR/immunohistochemistry	Harada <i>et al.</i> , 2002 (28)
	Aqueous humor (n=35)	ELISA	Shinoda <i>et al.</i> , 1999 (48)
	Vitreous (n=73)	ELISA	Katsura <i>et al.</i> , 1998 (47)
	Vitreous (n=41)	ELISA	Nishimura <i>et al.</i> , 1999 (98)
c-Met	Vitreous (n=17)	ELISA	Canton <i>et al.</i> , 2000 (96)
	Vitreous (n=27)	ELISA	Umeda <i>et al.</i> , 2002 (60)
	Vitreous (n=17)	ELISA	Briggs <i>et al.</i> , 2000 (97)
	Epiretinal membrane (n=3)	immunohistochemistry	Hollborn <i>et al.</i> , 2004 (99)
	Epiretinal membrane (n=3)	immunohistochemistry	Hollborn <i>et al.</i> , 2004 (99)
	Epiretinal membrane (n=30)	RT-PCR / immunohistochemistry	Harada <i>et al.</i> , 2002 (28)
	Vitreous (n=23)	ELISA	Freyberger <i>et al.</i> , 2000 (100)
	Aqueous humor (n=34)	ELISA	Funatsu <i>et al.</i> , 2001 (102)
	Vitreous (n=42)	ELISA	Abu El-Asrar <i>et al.</i> , 1997 (36)
	IL-8	Vitreous (n=30)	ELISA
Vitreous (n=47)		ELISA	Yuuki <i>et al.</i> , 2001 (104)

Table 1. (Contd.....)

ICAM-1	Vitreous (n=20)	ELISA	Esser <i>et al.</i> , 1995 (106)
	Vitreous (n=21)	ELISA	Barile <i>et al.</i> , 1999 (105)
	Vitreous (n=55)	ELISA	Limb <i>et al.</i> , 1999 (107)
VCAM-1	Vitreous (n=21)	ELISA	Barile <i>et al.</i> , 1999 (105)
	Vitreous (n=55)	ELISA	Limb <i>et al.</i> , 1999 (107)
	Vitreous (n=20)	ELISA	Hernandez <i>et al.</i> , 2001 (108)
TNF-	Epiretinal membrane (n=12)	immunohistochemistry	Limb <i>et al.</i> , 1996 (112)

ELISA, enzyme-linked immunosorbent assay; ISH, *in situ* hybridization; RT-PCR, reverse transcription-polymerase chain reaction.

has a chemotactic function for monocytes and induces monocyte and macrophage infiltration into tissues [32,33]. In addition, MCP-1 is a strong activator of monocytes and macrophages. These results suggest a possibility that MCP-1 is involved in the pathogenesis of DR. In fact, vitreous MCP-1 levels are increased in PDR compared with those in controls, and MCP-1 might recruit macrophages into the vitreous in PDR [24,34-36].

We have examined a relationship between vitreous MCP-1 levels and clinical grades of PDR. Multivariate regression analysis revealed a significant association between vitreous MCP-1 levels and the degree of proliferative ERM, and a significant negative association with MCP-1 levels and the extent of preoperative retinal photocoagulation [24]. Moreover, we examined the aqueous MCP-1 levels in all the stages of DR, and found that MCP-1 levels correlated with the clinical stage of DR from the nonproliferative stage [37]. These results suggest that MCP-1 may have a role in DR pathogenesis from the nonproliferative stage, and that chemokine receptors are potential targets for therapeutic intervention in DR.

Macrophage Migration Inhibitory Factor (MIF)

MIF is the first T-cell-derived soluble lymphokine reported to prevent random migration of macrophages and to recruit macrophages at inflammatory loci [38,39]. MIF is localized to astrocytes, Müller glial cells, and pigment epithelial cells in the rat retina [40]. Although its precise biological function remains undefined, MIF enhances macrophage adherence, motility, and phagocytosis [41]. These results suggest a possibility that MIF, like MCP-1, is involved in the recruitment of macrophages into eyes with DR. Therefore, we investigated the vitreous fluid of patients with PDR and reported that vitreous MIF levels were increased in PDR [22]. In addition, there is a significant association between MIF levels and grades of proliferative ERM [22]. Furthermore, retinal photocoagulation decreased MIF levels [22]. We also investigated the aqueous MIF levels in all the stages of DR, and found that MIF levels correlated with the clinical stage of DR from the nonproliferative stage [37]. These results suggest that MIF, as well as MCP-1, is involved in the pathogenesis of DR.

Vascular Endothelial Growth Factor (VEGF)

To date, VEGF is the most attractive candidate for being responsible for DR. VEGF is a member of a large family of angiogenic growth factors [42]. VEGF was first discovered as a vascular permeability factor, but subsequently recognized as an angiogenic factor and as a specific mitogen

for vascular endothelial cells [43]. VEGF also functions as a promoter of endothelial cell migration and an antiapoptotic, endothelial cell survival factor [15,44-46]. VEGF mRNA and proteins are expressed in numerous retinal cell types, including pericytes, astrocytes, Müller glial cells, and endothelial cells [47-50]. VEGF expression in cultured retinal microvascular endothelial cells is increased by high glucose-mediated oxidative stress [51]. It has been shown that ocular neovascularization is closely associated with increases in VEGF production. In animal experiments, injection of VEGF into the vitreous can induce preretinal and iris neovascularization [14]. Conversely, pathological vascular growth can be prevented by treatment with selective VEGF inhibitors [47,52-56]. In the monkey model of ocular neovascularization, the injection of soluble VEGF receptors suppressed iris neovascularization [57].

Consistent with the results from animal studies, many clinical studies have shown a strong correlation between increased intraocular VEGF concentration and the development of DR [47,48,58-62]. We also reported increased vitreous levels of VEGF protein in PDR and significant relationship between vitreous VEGF levels and PDR activity [25]. Moreover, VEGF and its receptors are localized to ERMs of PDR [63-65]. These results suggest that VEGF plays an important role in angiogenesis and ERM growth in DR. Furthermore, recent studies have shown that VEGF production and release from retinal cells is stimulated by other cytokines and trophic factors, which are considered to be involved in the pathogenesis of DR. Such functional interactions will be discussed in the following sections.

Placenta Growth Factor (PlGF)

PlGF is a close homologue of VEGF and shares its receptors with VEGF, but has little or undetectable mitogenic activity on endothelial cells. However, PlGF can potentiate the action of low concentrations of VEGF and indirectly stimulate endothelial cell proliferation, migration, and angiogenesis [43,46,66]. The study using PlGF gene inactivated mice indicated synergism between PlGF and VEGF in pathological angiogenesis and permeability [45]. The augmentation of VEGF action by PlGF may depend on the duration of PlGF exposure and on the origin of endothelial cells [46].

In PDR, PlGF levels were reported to be elevated in the retina [42], vitreous fluid [67], and ERMs [67]. We attempted to ascertain whether intravitreal PlGF levels correlate with VEGF levels or clinical activity in PDR. In our study, intravitreal PlGF levels were significantly correlated with VEGF levels [25]. Vitreous PlGF levels in

PDR were significantly higher than those in the controls. In addition, PIGF levels in active PDR patients were significantly higher than those in quiescent PDR patients [25]. Taken together, these results suggest that PIGF have a cooperative role with VEGF and is involved in the progression of PDR.

Tenascin-C

Tenascin-C is a large hexameric extracellular matrix glycoprotein, modulating cellular growth and adhesion. Tenascin-C is expressed in developing organs, during oncogenesis, wound healing, and inflammation [50]. Tenascin-C is also involved in the sprouting of endothelial cells, which is a necessary step in angiogenesis [20]. For example, tenascin-C is associated with vessel formation in granulation tissue and brain tumors [68,69]. Spirin *et al.* [42] reported that the major gene expression changes identified in retinas of DR, compared to normal retinas, concerns an increased expression of small tenascin-C isoforms, and described that increased tenascin-C contributes to the development of diabetic preretinal neovascularization and exacerbation of DR. We investigated the vitreous fluid of patients with PDR, and found higher concentrations of tenascin-C compared with control patients. In addition, intravitreal tenascin-C concentrations in active PDR patients were significantly higher than those in quiescent PDR patients [26]. Furthermore, tenascin-C was detected in ERMs from eyes with PDR [51,70]. These results implicate tenascin-C in the pathophysiology of PDR.

Spirin *et al.* [42] described a possibility that PDR development may be associated with increased retinal expression of VEGF and PIGF that possibly triggers the deposition of tenascin-C isoforms in the blood vessel walls. Angiogenesis-stimulating tenascin-C may further promote diabetic retinal neovascularization. Thus, extracellular matrix as well as cytokines may be a potential therapeutic target for DR.

Insulin-Like Growth Factor 1 (IGF-1)

IGF-1 is a polypeptide structurally homologous to insulin that regulates the proliferation and differentiation of several cell types [71]. Previous clinical studies have suggested the association between growth hormone/IGF-1 and the development of DR. Growth hormone deficient dwarfs with diabetes shows little evidence of DR [72]. In addition, PDR could improve after hypophysectomy [73]. Conversely, intravitreal injection of IGF-1 in rabbits or pigs causes a breakdown of the blood-retinal barrier and neovascularization that progress in a similar manner to PDR [74,75]. In human eyes, vitreous IGF-1 levels were significantly higher in PDR than in controls [71,76-78]. However, Gerhardinger *et al.* [79] recently reported a threefold lower IGF-1 mRNA levels in retinas obtained postmortem from diabetic human donors than in retinas of non-diabetic donors. Therefore, further studies are required to establish the precise role of IGF-1.

Basic Fibroblast Growth Factor (bFGF)

bFGF is a member of a large family of neurotrophic molecules existing in different protein isoforms (18, 21 and

22 kDa), which displays selective subcellular localization thus implying different functional roles [80,81]. bFGF binds to four related tyrosine kinase receptors (FGFR1-4), which exist in different splice variants [82]. bFGF supports the survival and maturation of both neurons and glial cells [81,83], and may play an important role in regeneration after neural injury [84]. Several laboratories have reported that both bFGF and its receptors are expressed in the retina, which can be modulated by different pathological conditions, including ischemic injury and photoreceptor degeneration [85-88]. These results suggest that bFGF can be rapidly modulated in the retina and might participate in short- and long-term adaptation under different situations. In addition, *in vivo* angiogenesis assays have revealed that bFGF is a potent angiogenic factor [89,90]. Exogenous administration of bFGF has been shown to induce endothelial proliferation and VEGF expression [91]. Consistent with these observations, bFGF concentration is increased in vitreous samples from patients with PDR [78,92]. These results suggest the important roles of bFGF in the pathogenesis of neovascularization in DR.

bFGF is also involved in the formation of ERM. Previous studies have shown high expression level of bFGF in ERMs of PDR [63,93]. We also examined bFGF expression in ERMs from control (idiopathic ERM) and PDR patients [28]. bFGF mRNA was detected in 10 of 15 (67%) nonvascular idiopathic ERMs, and in 13 of 19 (68%) ERMs from PDR patients. In 6 of 11 (55%) PDR samples, bFGF immunoreactivity was observed in a large area and partially double-labeled with glial cell markers. Although it was not specific for PDR, these results suggest an implication of bFGF in the formation of ERM, and that glial cells might have functional interactions with other cell types, such as endothelial cells. Similar findings were observed in hepatocyte growth factor (HGF) and glial cell line-derived neurotrophic factor (GDNF) receptors, which are discussed in the later sections.

Hepatocyte Growth Factor (HGF)

HGF and its receptor, the c-Met tyrosine kinase, play roles in the regulation of cell motility, cell growth, and morphogenesis of various cell types [94]. HGF has also been identified as a member of angiogenic growth factors, and c-Met is observed in vascular cells and cardiac myocytes. In addition, the mitogenic action of HGF on human endothelial cells was the most potent among growth factors. These results suggest a possibility that HGF is involved in the neovascularization in DR [95]. It was reported that HGF levels in aqueous humor were increased progressively in proportion to the stage of DR [48]. In addition, vitreous HGF levels were significantly higher in PDR than in controls [47,60,96,97]. Nishimura *et al.* [98] reported that PDR subjects with neovascularization of iris, which suggests advanced retinal ischemia, showed a higher mean vitreous HGF concentration than those without iris neovascularization.

Hollborn *et al.* [99] recently demonstrated that both HGF and c-Met receptors are expressed in glial cells in ERMs of PDR patients. Interestingly, in cultured retinal glial cells, bFGF evoked HGF secretion, and HGF promoted the

secretion of VEGF. As mentioned in the former section, VEGF can trigger the deposition of tenascin-C in the blood vessel walls that promote diabetic retinal neovascularization [42]. These results suggest an autocrine/paracrine role by HGF in glial cell responses during retinal neovascularization.

Other Cytokines

Apart from the factors described above, other cytokines or adhesion molecules such as platelet-derived growth factor (PDGF) [100], interleukine-6 (IL-6) [101,102], IL-8 [34,103,104], intercellular adhesion molecule-1 (ICAM-1) [105-107], and vascular cell adhesion molecule-1 (VCAM-1) [105,107,108] have also been reported to be increased in human vitreous in PDR. Although very low levels of tumor necrosis factor (TNF- α) are detected in the vitreous of PDR [109-111], TNF- α is observed predominantly in PDR ERMs [112].

Neurotrophins, Glial Cell Line-derived Neurotrophic Factor (GDNF) and their Receptors in ERMs

Members of the nerve growth factor (NGF) family of neurotrophins (brain-derived neurotrophic factors; BDNF, neurotrophin-3; NT-3, neurotrophin-4/5; NT-4/5) rescue retinal cells in animal models of retinal degeneration [85-88]. Control of cell survival by neurotrophins is mediated by two types of transmembrane glycoproteins, the trk tyrosine kinase receptors (trkA, trkB and trkC) and the neurotrophin receptor p75 (p75^{NTR}) [113]. Neurotrophins act in neural cell survival by activating trk tyrosine kinases, downstream of which a ras-dependent pathway leads to the activation of mitogen-activated protein (MAP) kinases [114,115]. Contrary to the action of neurotrophins on trk receptors, p75^{NTR} can induce apoptosis, and in fact this mechanism appears to be essential for developmentally regulated cell death in peripheral and central neurons [116]. We previously demonstrated that retinal degeneration alters expression levels of these neurotrophin receptors mainly in Müller cells, the principal glial cells in the retina [87,88]. Interestingly, exogenous neurotrophins modulate the production of secondary trophic factors, such as bFGF, in Müller cells by activating neurotrophin receptors [87,88]. These results suggest an indirect glia-neuron network during retinal degeneration. A similar network is observed in other trophic factor families. Glial cell line-derived neurotrophic factor (GDNF) is a member of the transforming growth factor- β (TGF- β)-related neurotrophic factor family. GDNF family members mediate their actions through a multicomponent receptor complex composed of a transmembrane tyrosine kinase receptor, Ret, and one of the four glycosyl-phosphatidyl inositol (GPI)-linked GDNF family receptors, designated GFR 1-GFR 4 [117]. We previously demonstrated that GFR 2 expression is upregulated in Müller cells during photoreceptor degeneration, and GDNF increases BDNF, bFGF, and GDNF production in Müller cells [118]. These results suggest that both NGF and GDNF families regulate phenotypic expression in Müller cells, and that their effects on surrounding neurons may be mediated through an indirect pathway.

Together with previous findings that glial cells are one of the main components of ERMs [119], NGF and GDNF

family members may be involved in the formation of ERMs. To determine the possibility, we examined the expression of receptors for neurotrophins (trkA, trkB, trkC, p75^{NTR}) and GDNF (GFR 1, GFR 2, Ret) in ERMs obtained from PDR and idiopathic ERM patients. Expression of neurotrophin receptors in PDR ERMs was stable compared with those in idiopathic ERM. However, GFR 2 mRNA expression in PDR ERMs was significantly higher than in idiopathic ERMs [28]. In addition, immunohistochemical analysis revealed the existence of GFR 2 protein in the glial component of PDR ERMs. These results demonstrate the possibility that GDNF is involved in the formation of the glial cell component of ERMs in PDR. Since GDNF increases bFGF production in Müller cells [118], released bFGF from retina and/or ERMs may stimulate endothelial proliferation and VEGF secretion [91], which accelerate the progression of DR. Together with the selective biosynthetic changes of Müller cells in human DR [120], inhibiting the enlargement of the glial component may be one of the promising strategies to prevent the development of fibrovascular ERMs.

TREATMENT

To date, laser photocoagulation is the main treatment for DR. However, diabetic patients are often asymptomatic until late in the course of the disease. When the patient notices a decrease in visual acuity, the optimal timing for laser treatment has usually passed. The number of PDR cases that suffer severe vision loss is estimated to be 10 to 20%, despite laser treatment [121]. Moreover, approximately 15% of patients have some mild or moderate visual side effects from the standard laser treatment for DR [122]. Therefore, a pharmacologic agent that is adjunctive to laser treatment and easily administered for prophylactic purposes would be helpful. The neovascularization in DR might be treated by growth factor blockade, extracellular matrix alteration (e.g., with steroids), or interference of intracellular signal transduction pathways. If neovascularization could be arrested at the early stage of DR, the following blinding complications would be avoidable. Numerous potentially useful anti-angiogenic drugs are in development. Some of these are presently in clinical trials for the treatment of PDR.

Recent studies have suggested a possibility that glial cells in ERMs produce and/or store growth factors, which activate vascular cells, and further stimulate neovascularization as well as the proliferation of other cell types [28,122]. In addition, we found the expression of nuclear factor kappa B (NF- κ B), a transcription factor that can be activated by proinflammatory cytokines, in the glial component of ERMs [123,124]. Thus, not only vascular endothelial cells, but also glial cells might be another important target for the prevention and treatment of fibrovascular ERMs in DR.

CONCLUSION

In DR, we and other groups have demonstrated increased concentration of cytokines and extracellular matrix in vitreous and/or aqueous humour, which may stimulate endothelial cells and trigger neovascularization. Moreover, there was a significant association between clinical grades of

DR and expression levels of these cytokines or extracellular matrix. We also examined trophic factor receptor gene expressions in ERMs of DR, and found high expression levels of GDNF receptor in the glial component. Therefore, we proposed a possible involvement of glial cells in the formation of vascular ERMs in DR. The revelation of these complex cytokine networks may lead to the new therapy utilizing specific blockers, neutralizing antibodies, and virus vectors. Further research to deepen our understanding of the precise pathogenesis of diabetes will produce widespread benefit in the management of DR.

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