Modulation of Thrombospondin 1 and Pigment Epithelium–Derived Factor Levels in Vitreous Fluid of Patients With Diabetes

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**Objective:** To determine the levels of 2 endogenous inhibitors of angiogenesis, thrombospondin 1 (TSP-1) and pigment epithelium–derived factor (PEDF), in the vitreous fluid from patients with and without diabetes.

**Methods:** The levels of TSP-1 and PEDF in vitreous samples from diabetic and age-matched nondiabetic patients were determined by Western blot analysis.

**Results:** We observed significant amounts of TSP-1 and PEDF in the vitreous samples of control eyes. The TSP-1 levels varied in samples from patients with diabetes. In contrast, PEDF levels showed little or no change in vitreous samples from patients with or without diabetes. However, the PEDF protein exhibited variation in its molecular weight among the samples. We consistently observed lower levels of TSP-1 in diabetic patients who expressed the higher-molecular-weight PEDF isoform.

**Conclusions:** In diabetes, changes in the TSP-1 level may play a role in shifting the angiogenic balance and contributing to the pathogenesis of diabetic retinopathy. Although the PEDF level did not change, the diabetic samples with the higher-molecular-weight PEDF isoform consistently showed lower levels of TSP-1.

**Clinical Relevance:** The presence of the higher-molecular-weight PEDF isoform may be associated with greater risk of severe diabetic retinopathy.


**Diabetic Retinopathy** is a serious microvascular complication and is a major cause of adult blindness when it progresses to the proliferative stage with active neovascularization. It is characterized by early microvascular damage and capillary nonperfusion resulting in retinal ischemia and retinal neovascularization. The retinal neovascularization is driven by ischemia, which results in increased production of several stimulators of angiogenesis and perhaps decreased production of inhibitors of angiogenesis. Thus, alterations in the balanced production of positive and negative regulators of angiogenesis may determine the pathogenesis of diabetic retinopathy. Many studies have focused on the role of positive factors such as vascular endothelial growth factor (VEGF). However, the potential role of the endogenous inhibitors of angiogenesis in the pathogenesis of diabetic retinopathy remains poorly understood.

Endogenous inhibitors of angiogenesis, including thrombospondin 1 (TSP-1) and pigment epithelium–derived factor (PEDF), which are present at ocular avascular sites such as vitreous, may play a key role in retinal vascular homeostasis. Thrombospondin 1 is a member of the thrombospondin family of the matricellular proteins with potent antiangiogenic activity. Thrombospondin 1 was the first identified endogenous inhibitor of angiogenesis whose expression was down-regulated during malignant transformation. We have shown that expression of TSP-1 is essential for appropriate development of retinal vasculature. Mice deficient in TSP-1 fail to undergo appropriate remodeling and pruning of the developing retinal vasculature and as a result exhibit increased retinal vascular density. We also observed high levels of TSP-1 in vitreous samples prepared from normal eyes of various species, including human, bovine, rat, and mouse. We showed that increased expression of TSP-1 in mouse eyes attenuates normal retinal vascular development and retinal neovascularization during oxygen-induced ischemic retinopathy. Thus, altered production of TSP-1 may play a significant role in the development and progression of diabetic retinopathy. The role of TSP-1 in the development and progression of diabetic retinopathy remains elusive. We previously showed that
TSP-1 is present at high levels in vitreous samples prepared from control rats, whereas it is absent in vitreous samples prepared from diabetic rats. This was associated with significant early vasculopathies observed in diabetic animals. We also showed that exposure of microvascular endothelial cells (ECs), including retinal ECs, to high glucose levels results in decreased production of TSP-1 and enhanced migration of retinal ECs. Furthermore, retinal ECs prepared from TSP-1–deficient mice maintain a proangiogenic phenotype in culture. Together, these studies indicate that TSP-1 plays a critical role in retinal vascular homeostasis as decreased production during diabetes may contribute to the pathogenesis of diabetic retinopathy. However, to our knowledge, the level of TSP-1 in the eyes of patients with diabetes has not been previously evaluated. Its contribution to the development and progression of diabetic retinopathy requires investigation.

Pigment epithelium–derived factor, a 50-kDa neurotrophic glycoprotein, is also an endogenous inhibitor of angiogenesis and may play a role in retinal vascular homeostasis. We recently showed that the development of retinal vasculature proceeds at a faster rate in PEDF–deficient mice and that the retinal vasculature is more sensitive to hyperoxia-mediated vessel obliteration during oxygen-induced ischemic retinopathy. This is in contrast to what we observed in the TSP-1–deficient mice, which exhibited protection from vessel obliteration in response to hyperoxia. Therefore, these molecules play in retinal vascular homeostasis are distinct and need further evaluation.

Like TSP-1, PEDF inhibits angiogenesis in a number of models and inhibits EC proliferation and migration in culture. It was previously reported that the vitreous level of PEDF is lower in patients with proliferative diabetic retinopathy compared with nondiabetic control subjects. In contrast, others have reported that the vitreous levels of PEDF are higher in diabetic patients with active proliferative retinopathy than in those with no neovascularization. Therefore, for improved management of diabetic retinopathy, a better understanding of the pathogenesis of this disease is crucial. The aim of this study was to examine whether alterations in TSP-1 and PEDF levels occur during diabetes.

**METHODS**

**PATIENTS**

Patients were recruited from the Department of Ophthalmology and Visual Sciences, University of Wisconsin School of Medicine and Public Health. Patients with diabetic retinopathy and age-matched control subjects were recruited from November 1, 2001, to July 31, 2003. The diagnosis of diabetes was made by the criteria of the American Diabetes Association reported in 1997. Patient history and duration of diabetes were gathered from medical records. All of the patients signed informed consent for their participation in the study. The study design and protocol were approved by the institutional review board of the University of Wisconsin School of Medicine and Public Health.

**RESULTS**

**BASELINE CHARACTERISTICS**

This study involved a total of 21 control subjects without diabetes who had a mean (SD) age of 69.7 (7.73) years and an idiopathic macular hole or epiretinal membrane (Table 1) as well as 35 diabetic patients with diabetic retinopathy.
retinopathy who had a mean (SD) age of 62.9 (7.57) years (Table 2). The mean (SD) duration of diabetes for diabetic patients was 22.2 (10.0) years. All of the diabetic patients had proliferative retinopathy. Sex and age were comparable between the diabetic and control groups (Table 1 and Table 2).

ALTERATIONS IN TSP-1 LEVEL IN VITREOUS SAMPLES FROM DIABETIC PATIENTS

We previously showed that TSP-1 is present at high levels in vitreous samples from normal rats, whereas it is significantly diminished with diabetes.8 We examined the level of TSP-1 in vitreous samples from all of the patients by Western blotting. The Figure shows a representative TSP-1 Western blot of vitreous samples from control subjects and diabetic patients as well as the quantitative assessment of the data. The data for the remaining samples are summarized in Table 1 and Table 2. Our results showed that a significant amount of TSP-1 is present in vitreous samples from control eyes. However, the level of TSP-1 in vitreous samples varied among patients with diabetes. To our knowledge, this is the first report of the detection of TSP-1 and its potential changes in vitreous samples from patients with diabetes.

LACK OF ALTERATIONS IN PEDF LEVEL IN VITREOUS SAMPLES FROM DIABETIC PATIENTS

We next determined whether the PEDF level changed in vitreous samples of diabetic patients compared with control subjects. The Figure shows a representative PEDF Western blot of vitreous samples from control subjects and diabetic patients. The results from the remaining samples are summarized in Table 1 and Table 2. We observed little or no differences in the level of PEDF detected in vitreous samples from diabetic patients compared with control subjects. However, PEDF exhibited a variation in its molecular weight among the samples (Figure, Table 1, and Table 2). Thus, our data suggest that in vitreous samples from control subjects and diabetic patients, there are 2 isoforms of PEDF that vary in their molecular weight. This may be owing in part to various polymorphisms previously reported in the human PEDF gene or its glycosylation.20 We consistently observed lower levels of TSP-1 in vitreous samples of diabetic patients with the higher-molecular-weight isoform of PEDF.

In this study, we analyzed the levels of 2 major endogenous inhibitors of angiogenesis, TSP-1 and PEDF, in the vitreous samples from patients with diabetes compared with those from control subjects. Our hypothesis is that decreased production of TSP-1 occurs during diabetes and contributes to the development and progression of diabetic retinopathy. We observed variation in the levels of TSP-1 in vitreous samples prepared from diabetic patients compared with control subjects. This ranged from minimal changes in TSP-1 levels to little or no detectable TSP-1 in vitreous samples from diabetic patients. In contrast, we detected significant levels of PEDF in all of

### Table 1. Characterization of Control Subjects

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<tr>
<th>Patient No./Sex/Age, y</th>
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<th>Duration of Diabetes, y</th>
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Many endogenous inhibitors of angiogenesis, including TSP-1 and its closely related family member TSP-2, angiostatin, endostatin, PEDF, interferon α/β, and platelet factor 4, have been reported. In patients with proliferative diabetic retinopathy, the tight regulation of angiogenesis is achieved by a balanced production of positive and negative factors. Alterations in this balance under various pathological conditions such as diabetes result in angiogenesis. Vascular endothelial growth factor acts as an EC mitogen in vitro and promotes vascular permeability and angiogenesis in vivo. Intravitreal VEGF concentrations increase during the periods of active intraocular neovascularization in patients with proliferative diabetic retinopathy. However, little is known about the potential changes in the expression and/or activity of endogenous inhibitors of angiogenesis during diabetes.

Many endogenous inhibitors of angiogenesis, including TSP-1 and its closely related family member TSP-2, angiostatin, endostatin, PEDF, interferon α/β, and platelet factor 4, have been reported.33,34 Thrombospondin 1 was the first identified endogenous inhibitor of angiogenesis whose decreased expression during progression of many solid tumors is associated with activation of the angiogenic switch. We consider TSP-1 to be an angiogenesis inhibitor associated with the development and progression of diabetic retinopathy. Thrombospondin 1 is present in vitreous and aqueous humor at high levels and is produced by almost all known cell types in the eye, including retinal ECs, astrocytes, and pericytes, and elsewhere, including retinal pigment epithelial cells, corneal epithelial cells and ECs, and trabecular meshwork.
cells.\textsuperscript{10,11,36-39} It specifically inhibits EC proliferation and migration and blocks angiogenesis and tumor growth.\textsuperscript{33,40} Thrombospondin 1 and its antiangiogenic peptides effectively inhibit new blood vessel growth during oxygen-induced ischemic retinopathy.\textsuperscript{9,44} However, the possibility that administration of TSP-1 and/or its antiangiogenic peptides may inhibit the development and progression of proliferative diabetic retinopathy needs evaluation. Production of TSP-1 and TSP-2 by astrocytes has recently been shown to be essential for appropriate synaptogenesis and retinal neuronal functions.\textsuperscript{42} Thus, alterations in TSP-1 levels may also affect retinal neuronal functions, contributing to visual dysfunctions associated with diabetes.\textsuperscript{43,44} Furthermore, decreased production of TSP-1, PEDF, and endostatin observed in eyes with age-related macular degeneration\textsuperscript{30,35} suggests an important role for these angiogenesis inhibitors in modulation of choroidal vascular homeostasis.

In diabetic retinopathy, the total protein concentration of the vitreous is generally increased perhaps owing to the loss of retinal EC barrier function, an early dysfunction associated with diabetes. Because change in the total vitreous protein content is a likely concern and an important early characteristic of diabetic retinopathy, we used volumes of vitreous rather than total protein concentration in our analysis to normalize across all of the samples. In addition, to circumvent the possibility of vitreous hemorrhage affecting our results, we removed cell debris and platelets, major sources of TSP-1, before further analysis of the samples. However, this does not rule out the potential release of TSP-1 from activated platelets, resulting in increased levels of TSP-1. Although obtaining vitreous samples from patients in early stages of diabetic retinopathy may be challenging, it will help to address these concerns. Alternatively, one can evaluate TSP-1 levels following laser treatment and quiescence of retinal vasculature. However, the limited changes observed in vitreous levels of PEDF suggest a minimal contribution from contaminating serum.

The TSP-1 concentration was unaffected in some diabetic patients and was dramatically down-regulated in others. Because we did not further classify the patients with diabetes into active (highly active neovascularization with rapidly proliferative membrane and fresh vitreous hemorrhage or retinal detachment) or quiescent (chronic neovascularization with extensive panretinal laser photocoagulation and minimal background retinopathy) groups, there are 3 possible explanations for the results seen here. First, diabetic retinopathy was active in patients with low TSP-1 levels in the vitreous fluid. Second, diabetic retinopathy may be quiescent in patients with a high level of TSP-1 in the vitreous fluid. Third, retinopathy may be active or inactive when the TSP-1 level is high or low, respectively, depending on the concentration of stimulators such as VEGF. Thus, the activity of diabetic retinopathy rather than its severity more accurately reflects the effects of angiogenic stimulators and inhibitors. Unfortunately, the lack of sufficient patient information was prohibitory in delineating these possibilities. Further investigation of VEGF levels and better classification of retinopathies are needed for more accurate assessment of the TSP-1 change during diabetes and its effect on the development and progression of diabetic retinopathy.

Pigment epithelium-derived factor is also a potent inhibitor of ocular angiogenesis.\textsuperscript{13} Alterations in PEDF levels in patients with diabetes compared with nondiabetic patients have been controversial and not clearly resolved. It was reported that the level of PEDF in vitreous was lower in patients with diabetes,\textsuperscript{20,21} There are also reports that the PEDF concentration was higher in the vitreous from patients with diabetes.\textsuperscript{22,23} Our study showed that PEDF was at consistently high levels in the eyes of diabetic patients and control subjects. However, we detected 2 different isoforms of PEDF in human vitreous samples with different migration or molecular weight (Figure). One isoform migrated more quickly than the other on sodium dodecyl sulfate–polyacrylamide gel electrophoresis. We consistently observed lower levels of TSP-1 from diabetic patients with the higher-molecular-weight PEDF isoform. For quantitative assessment of the PEDF levels in vitreous samples prepared from control subjects and diabetic patients were determined by Western blot analysis. A representative set of samples from control subjects and diabetic patients is shown. There is wide variation in the TSP-1 levels in samples from diabetic patients compared with control subjects. Minimal variation was observed in the PEDF level, but PEDF varied in its molecular weight among the samples. Figure. Determination of thrombospondin 1 (TSP-1) and pigment epithelium–derived factor (PEDF) levels in vitreous samples. A, The TSP-1 and PEDF levels in vitreous samples prepared from control subjects and diabetic patients were determined by Western blot analysis. A representative set of samples from control subjects and diabetic patients is shown. B, For quantitative assessment of the data shown in A, the TSP-1 band intensities relative to those of PEDF were determined as described in the “Methods” section.

Multiple polymorphisms in the human PEDF gene\textsuperscript{28} and an association between some of these polymorphisms (the promoter and exon 3 coding region) and diabetic retinopathy and age-related macular degenera-
ations in retinal vascular development and angiogen-
sis have been reported. However, it is not known
whether these polymorphisms result in production of pro-
teins with different molecular weights or expression lev-
els. The identity and the level of transcripts generated by
these polymorphisms as well as the identity and activity of
their potential products require further investigation.
A potential polymorphism reported in intron 5 (just up-
stream from the splice acceptor site) may be a potential
candidate for generation of an alternatively spliced tran-
script producing a protein of a different size.28 Thus, the
identity of the PEDF polymorphisms and their protein
products as well as their association with the severity of
diabetic retinopathy will be very informative. Further-
more, this may allow for the development of a screening
method for identification of diabetic patients who are at
greater risk for the development of proliferative diabetic
retinopathy.

As endogenous inhibitors of angiogenesis, TSP-1 and
PEDF exhibit different expression patterns and func-
tions in retinal vascular development and angiogen-
esis.29-31 Although the level of PEDF was not dramatically
changed in the eyes of patients with diabetes, the in-
volveinent of different PEDF isoforms in the pathogen-
esis of diabetic retinopathy needs further investigation,
especially in regard to their effect on TSP-1 expression.
It is possible that different isoforms of PEDF have dif-
ferent antiangiogenic potential during development and
progression of diabetic retinopathy, perhaps through
modulation of the TSP-1 level. Although vitamin A has
been shown to increase expression of TSP-1 and PEDF
in retinal pigment epithelial cells,46 nothing is known
about the regulation of TSP-1 by PEDF. Thus, further in-
vestigations are needed to address the relationship of pro-
angiogenic factors such as VEGF, isoforms of PEDF, and
TSP-1 expression in the pathogenesis of diabetic reti-
nopathy.

In summary, we have demonstrated that the vitreous
TSP-1 levels varied among diabetic samples and were con-
sistently lower in the diabetic patients who expressed the
higher-molecular-weight isoform of PEDF. The vitre-
ous levels of PEDF, however, did not vary significantly
among diabetic patients and control subjects. Thus,
decreased production of TSP-1 along with expression of the
high-molecular-weight isoform of PEDF in patients with
diabetes may indicate a greater risk of developing severe
retinopathies.

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REFERENCES

(8):839-841.
cient mice exhibit increased vascular density during retinal vascular develop-
ment and are less sensitive to hyperoxia-mediated vessel obliteration. Dev Dyn.
2003;228(4):630-642.
5. Huang Q, Wang S, Sorenson CM, Sheibani N. PEDF-deficient mice exhibit an en-
hanced rate of retinal vascular expansion and are more sensitive to hyperoxia-
6. Sheibani N, Frazier WA. Thrombospondin-1, PEDAM-1, and regulation of
7. Rastimajed F, Polverini PJ, Bouck NP. Regulation of the activity of a new inhibi-
8. Sheibani N, Sorenson CM, Cornelius LA, Frazier WA. Thrombospondin-1, a natu-
rional inhibitor of angiogenesis, is present in vitreous and aqueous humor and is
257-261.
production and neovascularization in transgenic mice over-expressing thrombo-
10. Huang Q, Sheibani N. High glucose promotes retinal endothelial cell migration
through activation of Src, PI3K/Akt/eNOS, and ERKs. Am J Physiol Cell Physiol.
11. Su K, Sorenson CM, Sheibani N. Isolation and characterization of murine retinal
12. Wang Y, Wang S, Sheibani N. Enhanced proangiogenic signaling in thrombo-
spondin-1-deficient retinal endothelial cells. Microvasc Res. 2006;71(3):143-
151.
retinopathy by the natural ocular antiangiogenic agent pigment epithelium-
epithelium-derived factor inhibits choroidal neovascularization. Invest Ophthal-
17. Kanda S, Mochizuki Y, Nakamura T, Miyata Y, Matsuyma T, Kanetake H. Pig-
ment epithelium-derived factor inhibits fibroblast-growth-factor-2-induced cap-
18. Mori K, Duh E, Gehlbach P, et al. Pigment epithelium-derived factor inhibits reti-
19. Chen L, Zhang SS, Barnstable CJ, Tombran-Tink J. PEDF induces apoptosis in
human endothelial cells by activating p38 MAP kinase dependent cleavage of mul-
20. Funatsu H, Yamashita H, Nakamura S, et al. Vitreous levels of pigment epithelium-
derived factor and vascular endothelial growth factor are related to diabetic macu-
21. Ogata N, Nishikawa M, Nishimura T, Mitsuyma Y, Matsuyma M. Unbalanced vitre-
ous levels of pigment epithelium-derived factor and vascular endothelial growth
22. Ogata N, Matsuoka M, Matsuyma K, et al. Plasma concentration of pigment epi-
and vascular endothelial growth factor (VEGF) in aged human choroid and eyes
25. Sun J, Williams J, Yan HC, Amin KM, Albeda SM, DeLisser HM. Platelet endo-
thelial cell adhesion molecule-1 (PECAM-1)-homomorphic adhesion is mediated by
immunoglobulin-like domains 1 and 2 and depends on the cytoplasmic domain and
polymorphism is associated with increased risk of wet age-related macular degeneration.
27. Iizuka H, Awata T, Osaki M, et al. Promoter polymorphisms of the pigment epi-

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