Objective: To evaluate the hypothesis that transforming growth factor β2 (TGF-β2) is involved in the cause of proliferative diabetic retinopathy (PDR).

Methods: We assayed TGF-β2 levels in the vitreous of patients with PDR and other vitreoretinal disorders. Forty-nine vitreous specimens were obtained from eyes of patients with PDR undergoing vitrectomy, and 19 vitreous specimens were obtained from eyes of patients with PDR and other vitreoretinal disorders. Forty-nine vitreous specimens from nondiabetic subjects served as controls. We assessed TGF-β2 levels using an enzyme-linked immunosorbent assay. Both mature and total TGF-β2 levels were quantified.

Results: The mean (± SD) total levels of TGF-β2 were 2634 (± 1652) pg/mL in the patients with PDR and 1305 (± 972) pg/mL in controls. The mean (± SD) levels of mature TGF-β2 were 244 (± 316) pg/mL in patients with PDR and 79 (± 81) pg/mL in controls. Total and mature TGF-β2 levels were significantly greater in patients with PDR (total TGF-β2, P < .001; mature TGF-β2, P < .01). Mature TGF-β2 levels were higher in the vitreous of patients who had severe fibrous proliferation.

Conclusion: The results indicate increased levels of both total and mature TGF-β2 in the vitreous of patients with PDR, suggesting that TGF-β2 plays an important role in the pathogenesis of PDR.

RESULTS

Mature TGF-β2 was detected in 46 of the 49 PDR specimens and in 16 of the 19 control specimens. After acid activation, TGF-β2 was detected in all specimens. The total TGF-β2 level in controls was 1305 (±972) pg/mL. The levels of TGF-β2 in controls were similar to those previously reported in vitreous specimens from eye bank eyes.19 In patients with PDR, the total level of TGF-β2 was 2634 (±1652) pg/mL. This was significantly greater than in the controls (P < .001) (Figure 1, left panel). The level of mature TGF-β2 was 79 (±81) pg/mL in controls and 244 (±316) pg/mL in patients with PDR, a significant difference (P < .01) (Figure 1, right panel). The total and mature TGF-β2 levels in the PDR group were analyzed with multiple regression by age, sex, duration of diabetes, extent of photocoagulation, and the glycosylated hemoglobin value. No significant correlations were found between the TGF-β2 levels and these factors. There was no correlation between TGF-β2 levels and the extent of VH or TRD. No difference was found in glycemic management. The mature-total ratio was 0.15 in the control group and 0.14 in the PDR group; not a significant difference. The levels of total and mature TGF-β2 were assayed with a specific-capture enzyme-linked immunosorbent assay kit (Amersham Life Science, Buckinghamshire, England). This immunoassay, using the quantitative “sandwich” enzyme immunoassay technique, detects only mature TGF-β2; latent TGF-β2 is not recognized by the antibodies in this kit. The 96-well plates provided were coated with murine antibody to human TGF-β2. After 50 µL of assay diluent was pipetted into each well, 200 µL of specimen dilutions or standards were incubated for 2 hours at room temperature. After washing, enzyme-linked polyclonal anti-TGF-β2 was added for detection and incubated for 2 hours at room temperature. After further washing, the substrate solution was added, and the reaction was stopped with sulfuric acid, 1 mol/L. The optical density was read at the 450-nm wavelength. Recombinant human TGF-β2, provided in the kit, was used as a standard. The standard curve showed a linear response in the range of 31.3 to 2000.0 pg/mL, using logarithm/logarithm paper. The TGF-β2 concentration was calculated by comparing specimen absorption with that of serial dilutions of human recombinant TGF-β2.

Before the assay, vitreous specimens were diluted 1:2 with the standard diluent provided in the kit. The assay detection limit was defined as 2 pg/mL. Given the specimen dilution, the lowest detectable level was 4 pg/mL. Most TGF-β2 found in intraocular fluid has been identified as the latent form.18 To assay total TGF-β2 (mature form plus the latent form), specimens had to be activated before the assay. Thus, to assay total TGF-β2, half the volume of each vitreous specimen was activated by acidification with a solution of hydrochloride, 150 mmol/L, for 30 minutes at room temperature, followed by sodium hydroxide neutralization before the assay.18 In our study, each specimen, therefore, was assayed both with and without activation.

STATISTICAL ANALYSIS

Data were statistically analyzed with the Mann-Whitney U test for comparison of the 2 groups and with the Kruskal-Wallis test for multiple groups. For statistical calculations, specimens with concentrations below the assay detection limit were entered as 4.0 pg/mL. The results were declared as significant at P < .05. Data are given as mean (±SD).
rubecotic glaucoma than in the group that had burned-out retinopathy; however, the difference was not significant (P = .26 for both).

According to the extent of retinal photocoagulation, the total TGF-β2 levels were 2276 (±1333) pg/mL in the NRP group, 2638 (±1632) pg/mL in the FRP group, 2704 (±1905) pg/mL in the PRP group, and 2818 (±1776) pg/mL in the sPRP group (Figure 2, left panel). The respective mature TGF-β2 levels were 485 (±324), 278 (±289), 124 (±112), and 139 (±125) pg/mL (Figure 2, right panel). There was no statistical difference among the 4 groups (mature TGF-β2, P = .06; total TGF-β2, P = .42), although there was a tendency toward a higher level of mature TGF-β2 in the NRP group preoperatively. The mature-total TGF-β2 ratios were 0.24 (±0.27) in the NRP group, 0.19 (±0.26) in the FRP group, 0.09 (±0.14) in the PRP group, and 0.11 (±0.17) in the sPRP group. No statistical difference was found among the 4 groups (total TGF-β2, P = .41), whereas mature TGF-β2 levels (right panel) were statistically different (P < .01). The vertical bars indicate mean ± SD.

Grouping by the degree of fibrous proliferation revealed the following: the total TGF-β2 levels were 2440 (±1426) pg/mL in the FPD group, 3084 (±1919) pg/mL in the FPA group, and 2091 (±1626) pg/mL in the FPP group (Figure 3, left panel). No significant difference among the 3 groups was detected (P = .07). The respective mature TGF-β2 levels were 133 (±106), 202 (±202), and 832 (±553) pg/mL (Figure 3, right panel). These 3 groups were statistically different (P < .01). When the mature TGF-β2 levels of the 4 groups were analyzed independently with each of the other groups, the levels in the FPP group were greater than in the other groups (FD group and FPA group, P < .01 for both).

**Clinical Findings of the Vitreous Specimens***

<table>
<thead>
<tr>
<th>Study Group</th>
<th>Diagnosis</th>
<th>Specimens, No.†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients with PDR (n = 48)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TRD with VH</td>
<td>Macular TRD with VH</td>
<td>16</td>
</tr>
<tr>
<td>Extramacular TRD with VH</td>
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<td>4</td>
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<tr>
<td>Macular TRD with retinal break and VH</td>
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<tr>
<td>TRD without VH</td>
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<td>15</td>
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<tr>
<td>Extramacular TRD</td>
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<td></td>
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<tr>
<td>Macular TRD with retinal break</td>
<td></td>
<td>4</td>
</tr>
<tr>
<td>VH (no TRD)</td>
<td>. . .</td>
<td>6</td>
</tr>
<tr>
<td>Others</td>
<td>Macular edema</td>
<td>2</td>
</tr>
<tr>
<td>Premacular hemorrhage</td>
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<td>1</td>
</tr>
<tr>
<td>Control subjects (n = 19)</td>
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</tr>
<tr>
<td>Idiopathic edema</td>
<td>9</td>
<td></td>
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<tr>
<td>Premacular hemorrhage</td>
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</tbody>
</table>

*PDR indicates proliferative diabetic retinopathy; TRD, tractional retinal detachment; VH, vitreous hemorrhage; and RD, retinal detachment.
†There were 49 eye specimens from 48 patients with PDR.
In the present study, we measured TGF-β1 levels in the vitreous fluid collected at the time of vitreous surgery from patients with PDR and other disorders (the control group). The total TGF-β1 level in patients with PDR was approximately 2 times greater and the mature TGF-β1 level was 3 times greater than in the controls. These findings are of interest in the study of PDR because TGF-β2 is known to be a growth factor with diverse functions, including those related to the formation of fibrous proliferative membranes and neovascularization.

The total TGF-β2 levels in the vitreous from patients with PDR was quantified by Boulton et al.21 In their study, total TGF-β2 levels in diabetic vitreous was not different from those in nondiabetic controls, but vitreous from the patients receiving insulin treatment contained TGF-β2 at relatively high concentrations when the diabetic group was subdivided according to glycemic management. This result did not agree with our findings. With regard to glycemic management, the dose of insulin administered to each patient may be related.

There have been several reports on the relation between TGF-β2 production and photocoagulation. Previous studies have reported that TGF-β2 levels were increased in rabbit vitreous and in the culture medium of retinal pigment epithelial cells after photocoagulation.20 21 Yoshimura et al22 reported that photocoagulated retinal pigment epithelial cells in vitro produced an inhibitor of endothelial cell proliferation and that the character of the inhibitor corresponded to that of TGF-β2. These studies suggest that TGF-β2 is involved in the suppression of neovascularization after retinal photocoagulation. According to these studies, the increase in TGF-β2 production after photocoagulation was considered to reach a fairly high level. The present study, however, showed no clear correlation between the extent of preoperative retinal photocoagulation and TGF-β2 concentration. The TGF-β2 level was measured early after photocoagulation in other studies, but in our clinical specimens, the time after photocoagulation varied widely, possibly resulting in inconsistencies in the findings. The increase in TGF-β2 concentration in the vitreous in patients with PDR in the present study may be a result of, not only the photocoagulation undergone preoperatively, but other factors also.

In this study, TGF-β2 levels were shown to increase in the vitreous of patients with PDR. This cytokine is thought to promote the pathogenic process of PDR by inducing the formation of proliferative membranes. The specific actions of TGF-β2 on neovascularization remain largely unknown; however, they are likely to be complex because the effects of this growth factor vary depending on its concentration, its interaction with the extracellular matrix, and the presence of other cytokines. Further investigations of these and other aspects of the mechanisms of action of TGF-β2 are needed to better understand the role of this molecule in PDR.

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