Role of the Intravitreal Growth Factors in the Pathogenesis of Idiopathic Epiretinal Membrane

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PURPOSE. The aim of the present study is to evaluate the roles of TGFs β1 and β2, gel cell line–derived neurotrophic factor (GDNF), and nerve growth factor (NGF) in the pathogenesis of idiopathic epiretinal membrane (ERM).

METHODS. Eight patients, six males and two females, with an average age of 60.25 ± 17.16 years (range, 33–75 years) who were affected by idiopathic ERM were enrolled in the study. All patients underwent standard pars plana vitrectomy surgery with membrane removal and specific ELISA was performed to evaluate TGFβ1, TGFβ2, GDNF, and NGF in the vitreous samples. This was repeated after acidification of the samples with hydrochloric acid.

RESULTS. Before acidification, ELISA analysis revealed a significant increase of TGFβ2 in the samples with idiopathic ERM (327.98 ± 99.58 pg/mL; range, 206.864 – 466.235 pg/mL) compared to the control group (187.17 ± 58.20 pg/mL; range, 132.758 – 271.707 pg/mL; t = 3.4; P < 0.05). A statistically significant difference was also obtained after acidification of the samples (618.15 ± 201.43 pg/mL; range, 409.795 – 866.215 pg/mL compared to 265.04 ± 98.15 pg/mL; range, 152.478 – 352.101 pg/mL; t = 4.5; P < 0.05). Notably, before acidification the differences in NGF between the two groups were not statistically significant (t = 0.79; P = 0.46), while after acidification a significant increase of the NGF levels in ERM samples was found in comparison with the control group (723.41 ± 235.4 vs. 242.84 ± 104.61; t = 3; P < 0.05).

CONCLUSIONS. The present study reveals that TGFβ2 and NGF are associated with idiopathic ERMs, suggesting a novel compensatory mechanism so far never proposed. (Invest Ophthalmol Vis Sci. 2011;52:5786–5789) DOI:10.1167/iovs.10-7116

The macular epiretinal membrane (ERM) is a pathology caused by a fibrocellular proliferation on the inner limiting membrane (ILM) followed by cellular contraction. ERM can be either idiopathic or secondary to vitreoretinal diseases, such as proliferative vitreoretinopathy (PVR), diabetic retinopathy, and intraocular inflammation. Contraction of the ERM causes a significant macular dysfunction, resulting in symptoms including metamorphopsia, severe visual reduction and, on occasion, central unilateral diplopia.1–3 Several studies suggest that posterior vitreous detachment (PVD) can damage the ILM, thereby permitting the migration of glial cells to the retinal surface. Another proposed hypothesis is that an incomplete PVD provides the conditions suitable for membrane proliferation in the adhesion area between the vitreous and the retina.1 Secondary ERM is frequently associated with retinal vascular disorders such as diabetic and hypertensive retinopathy and vascular occlusion. Nonetheless, it can be also caused by retinal tears, vitreal hemorrhages, retinal photocoagulation, ocular trauma, retinal vasculitis, uveitis, and surgery.3–6 The complete pathogenesis of ERM remains unknown. The cells involved in this process are RPE metaplastic cells, glial cells (Müller cells and astrocytes), hyalocytes, endothelial cells, fibroblasts, myofibroblasts, monocytes, and macrophages.3,4 Growth factors, cytokines, and the extracellular matrix are involved in cellular signal transmission and in tissue changes during the process of ERM formation. Interestingly, the main growth factors studied thus far in the pathogenesis of ERM are platelet-derived growth factor (PDGF), TGFβ1, FGF, and VEGF,7–9 although recently some authors have supposed that other growth factors could be involved in the pathogenesis of ERM.10,11 The aim of the present study was to assess the role played by TGFs β1 and β2, gel cell line–derived neurotrophic factor (GDNF), and nerve growth factor (NGF) in the pathogenesis of idiopathic ERM, because these have been recently proposed to contribute to the pathology.12–14 In detail, TGF β1 or β2 expression has been shown to be secondary in ERM, although there are few reports that show their involvement in the origin of idiopathic membranes.12 However, a recent and provocative study by Minchiotti et al.13 examined TGFβ1 and NGF activity in idiopathic ERM and revealed the increased expression of mRNA in both factors. No significant increase of NGF and TGFβ1 concentration has been observed in vitreous samples, while vitreous cells presented significant NGF mRNA hyperexpression in the face of a slight increase of TGFβ1 mRNA. Most notably, the expression of mRNA NGF receptors, such as p75-NTR and trkA-NGFR, was also significantly increased in the vitreous, whereas TGFβRII mRNA expression was clearly reduced compared to control cases.13 On the contrary, Harada et al.12 reported that the mRNA expression of p75-NTR and trkA-NGFR was modified in the examined samples. Such controversy highlighted the need for further and focused studies to clarify this subject.

Our study was conceived with this goal: define the role of growth factors—and especially of those that were, until now, unexploited—in the makings of a relevant pathology in mod-

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ern ophthalmology, and in this way delineate reliable markers to monitor its progression.

**METHODS**

Eight patients, six males and two females, with an average age of 60.25 ± 17.16 years (range, 33–75 years) who affected by idiopathic ERM were enrolled in the study. The ERM was diagnosed ophthalmoscopically and with Spectralis Optical Coherence Tomography (OCT) examination (Heidelberg Engineering, Heidelberg, Germany).

Exclusion criteria included a history or presence of other ocular diseases, a history of previous eye surgery, previous retinal tears or holes, diabetic retinopathy, retinal vein occlusion, and AMD. All patients underwent standard pars plana vitrectomy surgery with ERM removal. In the six cases, concomitant cataract surgery with intraocular lens implantation was needed. During the vitrectomy, vitreous samples of 500 μL were obtained.

As a control group, we used eight vitreous samples collected from eight patients, four males and 4 females, with an average age of 65.42 ± 15.78 years (range, 42–82 years) who underwent vitrectomy surgery for primary retinal detachment (within 72 hours of retinal detachment onset) and who had no sign of ERM.

All vitreous samples were kept at a temperature of -80°C. Then a specific ELISA (Enmax ImmunoAssay System; Promega, Madison, WI) with a minimum sensitivity of 32 pg/ml was performed to evaluate TGFβ1, TGFβ2, GDNF, and NGF in the vitreous samples. ELISA evaluation was repeated after acidification of the samples with hydrochloric acid. We followed this procedure because in the ELISA of the direct samples, the concentration of growth factors free in the extracellular matrix is measured. Acidification breaks the binding of the growth factors to the carrier proteins and to the membrane receptors. Therefore, the total growth factors (the part free in the extracellular matrix plus the part previously bound to the carrier proteins and to the membrane receptors) were measured. This study has been reviewed by the ethics committee of the University of Rome, and informed consent was obtained from the subjects after explanation of the nature and possible consequences of the study. The study followed the tenets of the Declaration of Helsinki.

The Student’s t test, linear regression, and correlation with Pearson’s coefficient were used for the statistical analysis. P < 0.05 was considered statistically significant.

**RESULTS**

On presurgery examination, every patient showed an ERM that was confirmed by Spectralis OCT. In every case, OCT revealed a hyperreflective ERM with macular edema, and retinal thickening. ELISA evaluation of the vitreous samples we performed revealed the following results: the mean concentration of TGFβ1 in the study group was 94 ± 47 pg/ml (range, 45–150 pg/ml), while in the control group this was 89 ± 21.7 pg/ml (range, 75–135 pg/ml; P = 0.5; Fig. 1a1). Even after acidification of the samples, no significant difference in the level of the growth factor was found (101 ± 55 pg/ml, [range 51-162 pg/ml] vs 93 ± 48 pg/ml, [range 77-139 pg/ml]; t = 2.4; P = 0.6; Fig. 1a2). However a significant increase of TGFβ2 was observed before acidification in the samples with hydrochloric acid. We followed this procedure because in the ELISA of the direct samples, the concentration of growth factors free in the extracellular matrix is measured. After acidification of the samples, no significant difference in the level of the growth factor was found (101 ± 55 pg/ml, [range 51-162 pg/ml] vs 93 ± 48 pg/ml, [range 77-139 pg/ml]; t = 3.4; P < 0.05; Fig. 1b1). A statistically significant difference was also obtained after acidification of the samples (618.15 ± 201.43 pg/ml, [range 409.795–866.215 pg/ml] vs 265.04 ± 98.15 pg/ml, [range 152.478–352.101 pg/ml]; t = 4.5; P < 0.05; Fig. 1b2). A univariate analysis of correlation (Pearson’s coefficient) between TGFβ2 levels and patients’ age was performed, which revealed a significant negative correlation (R = -0.825; P < 0.05). GDNF quantification indicated that there were no significant results with values below the ELISA sensitivity threshold of 32 pg/ml: 21.7 ± 6.25 pg/ml (range, 12-29 pg/ml) in the study group and 20.25 ± 4.3 pg/ml (range, 15-29 pg/ml) in the control group.

With the same methodology, NGF levels were also assessed (Fig. 1c1). The mean concentration of NGF in the study group was 87.5 ± 49.9 pg/ml (range, 17.24–138.32 pg/ml), while in the control group this was 60 ± 37.4 pg/ml (range, 7-214.8pg/ml; Fig. 1c1). Such a difference was anyway not statistically significant (t = 0.79; P = 0.46). However, after acidification a significant increase of NGF levels was found compared to the control group (723.41 ± 235.4 vs 242.84 ± 104.61; t = 3; P < 0.05; Fig. 1c2). Univariate analysis of correlation (Pearson’s coefficient) between NGF levels and patient age was performed and no negative correlation was observed (R = 0.6; P = 0.2).

**DISCUSSION**

The experimental evidence produced here indicates that levels of the two TGFβ isoforms, β1 and β2, differs within the vitreous with idiopathic ERM. The quantity of TGFβ1 was therefore similar to the control group, while the TGFβ2 levels were significantly higher in the vitreous of patients with idiopathic ERM. These results revealed the relative insignificance of TGFβ1 in the pathogenesis of idiopathic ERM despite previous studies that have reported an increase of TGFβ1 in the epiretinal membranes of PDR and PVR, even though no clinical and histopathological evidence has been produced to confirm an involvement of this factor in idiopathic ERM.13,15

Our results do indicate that TGFβ2 is associated with idiopathic ERM and possibly in its development. An increase of TGFβ2 has already been found in eyes with idiopathic ERM, PDR, and PVR, and several reports have shown a strong correlation between TGFβ2 levels and intraocular fibrosis.16–18 In light of this, it is reasonable to suppose that TGFβ2 could stimulate the differentiation of a specific type of glial cells into myofibroblasts (because no effect is recorded in astrocytes), inducing their contraction in the ERM. As reported by Khono et al.,19 cultured bovine hyalocytes present with a strong contractile activity of collagen gels and overexpression of α-smooth muscle actin (αSMA) in the presence of TGFβ2 at a concentration of 3 ng/ml. A similar scenario would determine ERM contraction together with induced hypercontraction of hyalocytes and their differentiation into myofibroblastic cells. According to the authors, the vitreous samples from idiopathic ERM patients presented a hypercontraction of collagen gels embedding cultured bovine hyalocytes—an effect that was almost completely inhibited in the presence of anti-TGFβ2 neutralizing the antibody already at a concentration of 1 mg/ml.19 The amount of TGFβ2 used by Khono et al. to induce hyalocytes’ contraction was 300 pg/ml,19 which is similar to the amount observed in the vitreous samples used in our study (327.98 ± 99.58 pg/ml compared to 285.34 ± 43.77). These data indicate that in idiopathic ERM, an amount of approximately 300 pg/ml may be sufficient to stimulate hypercontraction of hyalocytes and their differentiation into myofibroblasts.

A significant negative correlation between TGFβ2 concentration and age was observed, such that the youngest patients presented with higher levels of TGFβ2. Even though this evidence has not been previously reported, a similar correlation was described in human serum.20 An intimate cell signaling capacity associated with this mediator of cellular growth could be occurring and plausibly be stronger in younger subjects.
Although a role of the GDNF and its receptors was recently found in the proliferative vitreoretinal disorders, few studies address the association of this factor with idiopathic ERM. We also explored this possibility, but did not find any significant alteration, because both ERM samples and controls had a value below the sensitivity threshold of 32 pg/mL. An equal value was reported by Nishikori et al. In light of this, a suitable hypothesis could be that in retinal diseases, such as idiopathic ERM (our study samples), retinal detachment (our control samples), and macular hole (the control group in the study by Nishikori et al.), the vitreal GDNF amount is marginal, corroborating our findings that GDNF is not directly associated with idiopathic ERM.

Quite notably, we instead found a significant difference in the concentration of NGF after acidification of the samples. Although NGF levels before acidification were statistically similar between the two groups, acidification induced an elevation of its amount compared to the control group. The acidification process enables NGF protein to free itself from the bindings with carrier proteins and membrane receptors, thereby providing a true measurement of total NGF. Our results regarding NGF before acidification are in line with conclusions by Minchiotti et al., who pointed out that in the face of a reduced concentration of vitreal NGF, its mRNA expression was tangibly increased. This can be explained by our results, for which we hypothesize that in the pathologic vitreous, the levels of free NGF are low because most of the NGF proteins are likely to be bound to carrier proteins and membrane receptors of vitreal cells involved in the ERM development process. This would form the basis for an autocrine and possibly paracrine framework of signaling and justify the differences of NGF concentration observed before and after acidification. The response to NGF from vitreal cells could be therefore enhanced by two mechanisms: a rise in NGF intravitreal production and increased use of NGF with hyperexpression of membrane receptors that bind most of the available NGF proteins.

It is known that NGF could lead to the differentiation of myofibroblasts, and its receptors may therefore play a significant role in this process. This fundamental growth factor could therefore reprogram the function of the cells on the ERM and represent a means to diagnose the progress of the pathology.

Based on the evidence reported here, we can affirm that TGFβ2 is associated with the presence of idiopathic ERMs. This growth factor could stimulate some retinal cells to differentiate and therefore contract. Interestingly, production of TGFβ2 in
the ERM may be correlated with age and cell signaling, and could be greater in younger patients. More importantly, NGF seems to be associated with idiopathic ERM development via a fine mechanism of tuned regulation between bound and free factor: both increased expression and use of the factor could be involved in the intra- and intercellular signals that lead to ERM.

Nonetheless, at the same time, our data indicate that there is no evidence to suggest an involvement of TGFβ1 and GDNF in idiopathic ERM.

Additional investigations are essential to clarify the contribution of these different intravitreal growth factors in the pathogenesis of ERM and these will have to be based on a larger sample size. We believe that a novel means of diagnosis and suitable pharmacologic strategies may be developed to tackle the onset and progression of ERM.

Based on the evidence, we conclude that TGFβ2 and NGF do play a role in the pathogenesis of idiopathic ERM, and our future aim will be to characterize this in further detail.

References