Elevated M2 Macrophage Markers in Epiretinal Membranes With Ectopic Inner Foveal Layers

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PURPOSE. To analyze the differences in the vitreous cytokine profiles in epiretinal membrane eyes with and without an ectopic inner foveal layer (EIFL).

METHODS. Sixty eyes with epiretinal membrane (32 eyes without EIFL and 28 eyes with EIFL) were included. The vitreous samples were collected during surgery for epiretinal membrane. The cytokine levels of the vitreous were measured using a multiplex bead analysis.

RESULTS. The mean logMAR visual acuity was worse (0.42 vs. 0.37; P = 0.331) and the central foveal thickness was higher in the EIFL group (496.9 μm vs. 434.2 μm; P = 0.007) than they were in the group without EIFL. The mean EIFL thickness was 164.1 ± 67.7 μm in the EIFL group. On multiplex analysis of the vitreous cytokines, the levels of CD163 (21529 pg/dL vs. 10877 pg/dL; P = 0.002) and macrophage colony-stimulating factor (206 pg/dL vs. 159 pg/dL, P = 0.004) were significantly higher in the EIFL group than they were in the group without EIFL.

CONCLUSIONS. Eyes with EIFL had increased vitreous levels of M2 macrophage markers. The activation of glial cell proliferation by M2 macrophages may contribute to EIFL formation.

Keywords: epiretinal membrane, ectopic inner foveal layer, M-CSF, M2 macrophages, CD163

An epiretinal membrane (ERM) is a relatively common retinal condition that is characterized by fibrocellular proliferations on the inner retinal surface between the vitreous and retina.1-3 The term ERM covers a wide range of clinical entities. Some ERMs are associated with other retinal conditions, such as retinal vascular or inflammatory disease, tumors, retinal detachment, intraocular surgery, and trauma. In contrast, other ERMs are idiopathic.4 The severity of this disease varies largely between eyes. Therefore, various classification schemes have been proposed. The most widely used one was suggested by Gass in 1997.5

The introduction of SD-OCT has enabled clearer visualization of the ERM. SD-OCT has become the most powerful tool for both pre- and postoperative evaluation of ERM. These SD-OCT–derived findings, such as the central foveal thickness (CFT), the length of the photoreceptor outer segment, and the inner segment ellipsoid band integrity, were found to be prognostic factors for ERM.6,7 Recently, ectopic inner foveal layer (EIFL) was described on SD-OCT imaging of eyes with idiopathic ERMs.8 This eccentric layer of inner retina at the fovea may represent an important sign of ERM progression. The EIFL was associated with significant vision loss.9,10 Therefore, a novel SD-OCT–based grading scheme of ERMs, including this finding, was suggested.8

The precise pathophysiology of ERM and EIFL is not completely understood. However, the mechanism likely involves hyalocyte proliferation in the setting of anomalous posterior vitreous detachment.4 The purpose of this study was to compare the vitreous cytokine profiles in patients with idiopathic ERMs with and without EIFL. We focused on the degree to which fibrotic and inflammatory processes contribute to the progression of the disease.

METHODS

Patients

This prospective clinical study included idiopathic ERMs in patients who underwent vitrectomy at Uijeongbu St. Mary’s Hospital (Gyeonggi-do, Korea), the Catholic University of Korea, between March 1, 2016, and September 30, 2017. The study was approved by the institutional review board/ethics committee of the Catholic University of Korea. Consent was obtained from all patients. The data were prospectively collected from the electronic medical records. The inclusion criteria were idiopathic ERM with epiretinal membranes by SD-OCT and visual acuity worse than 0.3 logMAR. The exclusion criteria were uncontrolled diabetes mellitus or hypertension, macular pucker, macular hole, subretinal fluid, and history of intraocular surgery.
The presence of continuous hyporeflective and hyperreflective bands extending from the inner nuclear layer and inner plexiform layer across the foveal region. The measurement of EIFL thickness was performed manually using the caliper function in the Heidelberg software. This function drew a vertical line from the outer margin of the inner nuclear layer to the inner margin of the internal limiting membrane at the center of the fovea on a 1:1 mm ratio image.

**Results**

**Baseline Clinical Characteristics**

A total of 60 eyes from 60 patients with idiopathic ERM were enrolled in this study. The mean age was 69.58 ± 10.30 years (range, 57–89 years). Nineteen patients (32%) were male. The mean logMAR BCVA was 0.45 ± 0.32 (with a Snellen equivalent of 20/56). The mean CFT was 464.45 ± 93.24 μm. EIFLs were present in 28 eyes (47%) (EIFL group); 32 eyes (53%) did not show EIFL (non-EIFL group). The baseline characteristics of the study subjects are summarized in Table 1.

**Comparison of Clinical Characteristic Between EIFL and Non-EIFL Eyes**

The mean thickness of the EIFL was 164.14 ± 67.69 μm in the EIFL group. The mean age did not differ between the
| Clinical Factors                              | Total (N = 60) | Non-EFL Group (n = 32) | EFL Group (n = 28) | P Value |
|----------------------------------------------|----------------|------------------------|--------------------|---------|
| Sex (male:female)                            | 19:41          | 10:22                  | 9:19               | 0.877   |
| Age (years)                                  | 69.58 ± 10.30 (57-89) | 68.06 ± 10.38 | 71.21 ± 7.85 | 0.192   |
| BCVA (logMAR)                                | 0.45 ± 0.32    | 0.37 ± 0.35            | 0.42 ± 0.32        | 0.032   |
| CFT (µm)                                     | 463.45 ± 93.24 | 434.15 ± 99.40        | 496.92 ± 73.99     | <0.001  |
| EFL thickness (µm)                           | 164.14 ± 67.69 | 0                    | 164.14 ± 67.69     | 0.581   |
| Presence of ellipsoid disruption, n (%)      | 32 (54)        | 16 (50)                | 16 (57)            | 0.581   |
| Presence of ELM disruption, n (%)            | 13 (21)        | 5 (15)                 | 8 (28)             | 0.225   |

**Table 1.** Baseline Clinical Characteristics

| Cytokines                        | Non-EFL Group (n = 52) | EFL Group (n = 28) | P Value |
|----------------------------------|------------------------|--------------------|---------|
| MCP-1 (CCL2) (pg/mL)             | 469.37 ± 305.56        | 701.84 ± 384.1     | 0.011*  |
| MCP-1beta (CCL4) (pg/mL)         | 92.22 ± 21.15          | 97.63 ± 21.67      | 0.351   |
| GRO alpha (CXCL1) (pg/mL)        | 43.03 ± 23.72          | 34.93 ± 30.83      | 0.251   |
| IP-10 (CCL10) (pg/mL)            | 35.02 ± 34.34          | 39.25 ± 29.85      | 0.457   |
| SDF-1 alpha (CXCL12) (pg/mL)     | 387.49 ± 159.29        | 396.69 ± 180.74    | 0.835   |
| IL-8 (CXCL8) (pg/mL)             | 17.13 ± 21.23          | 15.29 ± 12.53      | 0.689   |
| GM-CSF (pg/mL)                   | 1.09 ± 1.43            | 1.68 ± 1.98        | 0.184   |
| IFN-gamma (pg/mL)                | 2.15 ± 2.88            | 3.62 ± 5.11        | 0.164   |
| IL-17A (pg/mL)                   | 2.12 ± 0.53            | 2.27 ± 0.65        | 0.342   |
| IL-18 (pg/mL)                    | 9.61 ± 3.24            | 10.26 ± 4.47       | 0.513   |
| IL-2 (pg/mL)                     | 230.76 ± 240.11        | 190.65 ± 60.72     | 0.393   |
| IL-4 (pg/mL)                     | 1.35 ± 5.23            | 10.61 ± 27.22      | 0.060   |
| CD163 (pg/mL)                    | 10876.69 ± 9558.28     | 21528.89 ± 15322.98| 0.002*  |
| M-CSF (pg/mL)                    | 158.52 ± 63.87         | 206.13 ± 58.03     | 0.004*  |
| MMP-9 (pg/mL)                    | 61.00 ± 53.03          | 70.9 ± 65.6        | 0.517   |
| Periostin (pg/mL)                | 0 ± 0                  | 139.35 ± 737.38    | 0.281   |
| VEGF (pg/mL)                     | 9.50 ± 21.87           | 9.37 ± 29.21       | 0.984   |

GM-CSF, granulocyte-macrophage colony-stimulating factor; GRO, growth-related oncogene; IP, interferon gamma-induced protein; MCP, monocyte chemoattractant protein; MMP, matrix metalloprotease; SDF, stromal cell-derived factor.

*Statistically significant P value.
FIGURE 2. Correlation between M-CSF and CD163 in the non-EIFL group and EIFL group. The levels of CD163 and M-CSF were negatively correlated in the EIFL group ($r = -0.476; P = 0.011$) while no significant correlation was observed in the non-EIFL group ($r = 0.050; P = 0.781$).

TABLE 3. Correlation of CD163/M-CSF and Other Cytokines in the Non-EIFL and EIFL Groups

| Cytokines       | Non-EIFL Group ($n = 32$) | EIFL Group ($n = 28$) |
|-----------------|---------------------------|-----------------------|
|                 | CD163         | M-CSF         | CD163           | M-CSF           |
| MCP-1           | r = 0.807     | M = 0.034     | r = 0.828       | M = -0.284      |
|                 | $P = 0.000^*$ | $P = 0.850$   | $P = 0.000^*$  | $P = 0.143$     |
| MIP-1beta       | r = 0.494     | M = 0.302     | r = 0.418       | M = -0.082      |
|                 | $P = 0.005^*$ | $P = 0.087$   | $P = 0.027^*$  | $P = 0.677$     |
| IP-10           | r = 0.514     | M = -0.126    | r = 0.923       | M = -0.593      |
|                 | $P = 0.002^*$ | $P = 0.484$   | $P = 0.001^*$  | $P = 0.012^*$   |
| SDF-1alpha      | r = -0.311    | M = 0.185     | r = -0.696      | M = 0.469       |
|                 | $P = 0.078$   | $P = 0.302$   | $P = 0.000^*$  | $P = 0.011^*$   |
| IL-18           | r = 0.486     | M = -0.159    | r = 0.618       | M = -0.468      |
|                 | $P = 0.004^*$ | $P = 0.378$   | $P = 0.001^*$  | $P = 0.012^*$   |
| GM-CSF          | r = 0.416     | M = -0.060    | r = 0.376       | M = -0.461      |
|                 | $P = 0.016^*$ | $P = 0.740$   | $P = 0.048^* $ | $P = 0.014^*$   |
| IL-18           | r = 0.395     | M = -0.097    | r = 0.225       | M = -0.324      |
|                 | $P = 0.023^*$ | $P = 0.591$   | $P = 0.249$    | $P = 0.092$     |
| IL-4            | r = 0.079     | M = -0.348    | r = 0.408       | M = -0.401      |
|                 | $P = 0.661$   | $P = 0.047^*$ | $P = 0.031^*$  | $P = 0.035^*$   |
| MMP-9           | r = 0.615     | M = -0.021    | r = 0.447       | M = -0.062      |
|                 | $P = 0.000^*$ | $P = 0.908$   | $P = 0.017^*$  | $P = 0.752$     |

GM-CSF, granulocyte-macrophage colony-stimulating factor; GRO, growth-related oncogene; IP, interferon-gamma-induced protein; MCP, monocyte chemoattractant protein; MMP, matrix metalloprotease; macrophage inflammatory protein; SDF, stromal cell-derived factor.

a Pearson correlation.

b Two-tailed significance.

* Statistically significant $P$ value.

with and without EIFL can serve as evidence of ERM progression involving EIFL.

Since the introduction of SD-OCT, there is an increasing number of SD-OCT studies that have investigated alterations in the retinal layers in idiopathic ERM. These studies have shown that the outer retinal parameters, such as ellipsoid zone disruption and photoreceptor outer segment length, have been associated with lower preoperative and postoperative visual acuity.14–17 More recently, the role of the inner retinal layers in visual acuity loss has been studied. Factors such as the ganglion cell–inner plexiform layer complex thickening and the inner retinal irregularity index have been introduced as valid tools to assess visual prognosis.18–20 One of the most important recent findings associated with BCVA in ERM is EIFL. Govetto et al8,10 demonstrated that the presence of EIFLs in ERMs is associated with significant vision loss in these eyes. It is also a negative prognostic factor for postoperative anatomic and functional recovery. The baseline BCVA in this study is in accordance with previous reports. Eyes with EIFLs have worse BCVA compared to eyes without EIFL (logMAR 0.42 μm vs. 0.37 μm and logMAR 497 μm vs. 434 μm, respectively).

The pathogenetic mechanism for EIFL is not yet fully understood. It is hypothesized that EIFL is caused by mechanical displacement of the inner retinal tissue driven by ERM.21 Other investigators have suggested that Muller cells are involved at the molecular level.22 The analysis of vitreal cytokines in the current study revealed that CD163 and M-CSF were significantly higher in the EIFL group than they were in the non-EIFL group. Elevated M-CSF levels reflect macrophage differentiation. CD163 is a surface marker of M2 macrophages.22,23 Therefore, these results imply that M2 macrophages may play an important role in EIFL formation.

M-CSF stimulates the survival, proliferation, and differentiation of mononuclear phagocytic cells from determined (but undifferentiated) monoblasts to mature macrophages.22 The elevation of this cytokine in eyes with EIFL suggests active differentiation of monocytes into M2 macrophages. The M-CSF-differentiated M2 are polarized into activated...
M2 macrophages by IL-4. These cells express the M2 cell-surface marker CD163. Although it failed to reach statistical significance, the IL-4 level was elevated in the EIFL group compared to that in the non-EIFL group in this study (10.61 pg/mL vs 1.35 pg/mL; \( P = 0.060 \)). The activated M2 macrophages participate in constructive processes like wound healing and tissue repair. These macrophages are the phenotype of resident tissue macrophages. EIFL might be the fibrotic tissue that results from the wound healing process of the M2 macrophage.

CD163 is known to be a highly specific monocye/macrophage marker for M2 macrophages. In the vitreous, hyalocytes are known to be immunoreactive CD163. Hyalocytes belong to the monocye/macrophage lineage. Along with CD45, CD163 presents predominantly in the ERM in eyes with complete posterior vitreous detachment and vitreomacular traction. The increased level of CD163 in the vitreous of the EIFL group might reflect an increased number or increased activity of hyalocytes in these eyes. Hyalocytes are the phenotype resident tissue M2 macrophages, which can be further activated by IL-4. The positive correlation between CD163 and IL-4 in eyes with EIFL suggests that there is a positive feedback relationship between IL-4 elevation and increased hyalocyte activity (as a M2 macrophage). Further studies using histology or cytology are needed to further evaluate the other cells involved in the fibrotic processes in the retina.

M-CSF and CF163 were found to be positively correlated in the vitreous of eyes with proliferative diabetic retinopathy. The positive correlation between these two cytokines was also identified in the peritumor area of hepatocellular carcinoma. In contrast, there was a negative correlation between M-CSF and CD163 in eyes with EIFL eyes in the current study. In contrast, there was no significant correlation between M-CSF and CD163 in the non-EIFL eyes. We cautiously expect that the fibrotic process involving M2 macrophages might not be as active as in proliferative diabetic retinopathy or in tumors. There may be a controlling mechanism that decreases fibrosis. The negative correlation between M-CSF and IL-4 also suggests the presence of a negative feedback mechanism in the differentiation and activation of M2 macrophages.

This study has several limitations. First, the cytokine levels were measured from the vitreous; therefore, they may not represent the cytokine levels at the lesion site. In addition, the study interpretation may have been limited by its small sample size. Further, larger studies that address lesion histology are required to substantiate our results. However, we believe that this study is valuable because it is the first one to reveal cytokine profiles related to EIFL in eyes with ERM.

In summary, eyes with EIFL represent a severe form of ERM with regard to the baseline visual acuity and postoperative outcomes. We suspect the increased expression levels of M-CSF and CD163 reflect that the M2 macrophages play a role in the formation of EIFL in eyes with ERM. The healing process of M2 macrophages causing fibrosis seems to contribute to the formation of EIFL in eyes with ERM, and this may result in worse outcome after the surgery in these eyes. However, the molecular mechanisms that regulate M2 macrophage polarization, and the detailed process involving activated M2 macrophage in the formation of EIFL have not been determined. Further studies are needed to identify the processes that are involved in EIFL development.

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