

# 学位論文の要旨

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学位論文名      Multiplex Cytokine Analysis of Aqueous Humor in Eyes  
With Primary Open-Angle Glaucoma, Exfoliation Glaucoma,  
and Cataract

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## 論文内容の要旨

### INTRODUCTION

Glaucoma is characterized by a progressive “glaucomatous” optic neuropathy and corresponding visual field loss, in which elevated intraocular pressure (IOP) is the main risk factor. In open-angle glaucoma (OAG) including primary open-angle glaucoma (POAG) and exfoliation glaucoma (EXG), the IOP increases because of reduced aqueous humor outflow at the trabecular meshwork (TM). This results from increased aqueous humor outflow resistance due to changes in the amount and quality of the extracellular matrix (ECM) in the TM. Although the involvement of various genetic and internal/external stress factors such as immune reactions, inflammation, ischemia, hypoxia, and oxidative stress, have been proposed, the exact mechanism of the ECM changes in POAG and EXG is still not well understood. If an immune reaction and/or chronic inflammation at the TM or anterior segment is associated with elevated IOP in glaucoma, changes in ocular inflammatory cytokine expression might be detectable as changes in cytokine concentrations in the aqueous humor, because of the cytokine autocrine and paracrine mechanisms of action. Until now, several studies that measured cytokine concentrations in aqueous humor samples of eyes with glaucoma have detected elevated cytokine levels using traditional enzyme-linked immunosorbent assay (ELISA) techniques. A major limitation of testing aqueous humor is that only small sample

volumes (typically 50-150  $\mu$ l of fluid) can be obtained from human eyes; these amounts are barely sufficient to test a few cytokines using traditional ELISA techniques. Multiplex bead immunoassays allow for simultaneous detection of multiple cytokines in small volume clinical samples, have been used to determine cytokine expression profiles in the aqueous humor of patients with uveitis and branch retinal vein occlusion. A recent study used this technique to identify cytokine levels in aqueous humor obtained primarily from eyes with POAG. In the current study, to investigate the possible roles of the cytokine network in the pathological mechanism of glaucoma, we measured multiple cytokines related to inflammation in the aqueous humor of eyes with POAG, EXG, and cataract using a multiplex bead immunoassay technique.

### **MATERIALS AND METHODS**

Aqueous humor samples were obtained from 64 eyes of 64 Japanese subjects (POAG, 20 eyes; EXG, 23 eyes; and cataract, 21 control eyes). Aqueous humor samples (100 to 200  $\mu$ l) were aspirated at the beginning of glaucoma or cataract surgery with care taken to prevent blood and intraocular tissue contamination, immediately frozen and stored at  $-80^{\circ}\text{C}$  until the analyses were performed. The levels of eight cytokines including interleukin (IL) 1- $\beta$ , IL-6, IL-8, transforming growth factor (TGF)- $\beta$ 1, tumor necrosis factor (TNF)- $\alpha$ , serum amyloid A (SAA), migration inhibitory factor (MIF), and vascular endothelial growth factor (VEGF)-A were estimated using the multiplex bead immunoassay system.

### **RESULTS AND DISCUSSION**

All cytokines were detected in more than 90% of the samples analyzed in each group, except for SAA in the cataract group in which five (24%) of the 21 samples were below the detection limit of the assay and TNF- $\alpha$  in the EXG group in which three (13%) of the 23 samples were below the detection limit. Compared to the cataract group, the levels of TGF- $\beta$ 1, IL-8, and SAA were significantly higher in aqueous humor samples from the POAG (5.0-fold, 2.3-fold, and 11.9-fold, respectively) and EXG (12.5-fold, 4.0-fold, and 18.3-fold, respectively) groups. Except for a significant decrease in the IL-6 level in the POAG (0.23-fold) group, no other cytokine levels differed in the POAG and EXG groups compared with the cataract group. The levels of TGF- $\beta$ 1, IL-8, and SAA were positively correlated with each other ( $\rho=0.723-0.786$ ,  $P<0.0001$ ), the intraocular pressure (IOP) ( $\rho=0.392-0.662$ ,  $P<0.0001-0.0019$ ), and the number of glaucoma medications ( $\rho=0.478-0.659$ ,  $P<0.0001-0.0001$ ).

Using the bead immunoassay, we found clear elevations of cytokines related to an

immune reaction or inflammation compared to control eyes, that is, IL-8, TGF- $\beta$ 1, and SAA in the aqueous humor samples from the POAG and EXG groups. Previously, an elevated concentration of TGF- $\beta$ 1 was detected using a conventional ELISA technique in aqueous humor samples from patients with pseudoexfoliation syndrome (PXS) and EXG, and IL-8 by bead immunoassay in aqueous humor from OAG, suggesting good reproducibility of the multiplex bead immunoassay in the current study. This is one of the earliest studies to report successful detection of multiple cytokines using the multiplex bead immunoassay in aqueous humor from POAG and EXG.

In addition, the significant correlations among the TGF- $\beta$ 1, IL-8, and SAA levels and the significant correlations of these cytokines and the IOP levels in the present study suggested that the cytokine networks play important roles in IOP elevations in OAG, although the exact mechanism of the interactions among these cytokines are unclear.

### **CUNCLUSION**

Cytokine networks including TGF- $\beta$ 1, IL-8, and SAA in aqueous humor may have critical roles in IOP elevations in patients with OAG.