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Novel inflammatory cytokines (IL-36, 37, 38) in the aqueous humor from patients with chronic primary angle closure glaucoma

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ABSTRACT

Glaucoma eventually leads to optic nerve damage and vision loss without medical intervention. More than 50% of glaucoma caused blindness are attributed to primary angle closure glaucoma, particularly in Asians. It is reported that immune inflammation is involved in the progress of glaucoma.

Increased inflammation cytokines are detected in the aqueous humor of chronic primary angle closure glaucoma (CPACG). IL-36, IL-37 and IL-38, are novel cytokines and are involved in many inflammatory diseases, including inflammatory bowel diseases and acute anterior uveitis, but the possible contributing role in the pathogenesis of CPACG is unclear. In our current study, increased IL-36, IL-37 and IL-38 were detected in the aqueous humor of CPACG compared with age-related cataract (ARC). Furthermore, a significant correlation was detected between mean deviation of visual field (MDVF) of CPACG and IL-36, IL-37 or IL-38, respectively. Our data suggest IL-36, IL-37 and IL-38 might contribute to the immunological mediated pathogenesis of CPACG, despite the eye being an immune-privileged organ under normal conditions. The precise underlying mechanism of these cytokines during the development of CPACG remains to be explored. Our findings may be useful in therapeutic targeting of specific pathology.

1. Introduction

Glaucoma leads to optic nerve damage eventually and vision loss without medical intervention, mainly due to pathologically high intraocular pressure (IOP). Chamber angle closure may impair aqueous humor outflow through the trabecular meshwork, resulting in an elevated IOP and subsequently damage to the optic nerve and loss of visual function. More than 50% of glaucoma caused blindness cases are attributed to primary angle closure glaucoma (PACG), particularly in Asians [1]. Furthermore it has been demonstrated that there is up-regulation of some pro-inflammatory cytokines, including IL-6, IL-8, G-CSF, MCP-1, MCP-3 and VEGF in the aqueous humor from acute primary angle closure glaucoma (APACG) [2]. The observation that increased pro-inflammatory cytokines occur in the APACG eyes suggests that there is immune mediated inflammation involved in the aqueous humor of APACG. This is supported by the finding that anterior chamber inflammation is also detected in chronic glaucoma, including increased pro-inflammatory mediators, e.g. exotoxin, MIP-1β and IP-10 [3]. Increased inflammation in chronic primary closure glaucoma (CPACG), even in these patients without clinical evidence of inflammation, strongly suggests a possible role for inflammatory cytokines during the development of CPACG [3]. Interestingly, glaucoma patients exhibit continuous progression of neurons loss and visual field (VF) deterioration, despite IOP being under control [4,5], suggesting the pathogenesis of glaucoma is beyond pressure-mediated damage and perhaps is involved in host immunity/inflammation [6]. The possible explanation of such a condition is that pathophysiological stress due to elevation of IOP damages the optic nerve, and subsequently triggers secondary immune or autoimmune responses, and eventually leads to retinal ganglion cells (RGCs) and axon damage [6].

It is reported that modified IL-1 family profile cytokines, including IL-36, IL-37 and IL-38, can be found in the aqueous humor and sera of patients with HLA-B27 associated acute anterior uveitis and idiopathic acute anterior uveitis, compare to the age-related cataract (ARC) patients [7,8]. IL-36, IL-37 and IL-38 involve in inflammatory diseases, including inflammatory bowel diseases [9], arthritis [10] and

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atherosclerosis [11]. However it is unclear if there is modification of production of IL-36, 37 or 38 in CPACG and possible involvement of these cytokines in the development of CPACG. Based on the observations mentioned above concerning the involvement of IL-36, 37 or 38 in inflammatory mediated diseases, it is reasonable to speculate that such cytokines may also contribute to the pathogenesis of CPACG.

In the current study, we aimed to determine if there is altered production of IL-36, IL-37, and IL-38 in the aqueous humor of eyes from CPACG compared to ARC. Our data may be useful in therapeutic targeting of precise treatment of glaucoma.

2. Methods

2.1. Subjects and enrollment criteria

This prospective study was performed in accordance with the protocols of the Declaration of Helsinki and approved by the Clinical Research Ethics Committee at the Fudan University affiliated Huashan Hospital. Informed consent was obtained from each of participant prior to the enrollment.

All eyes of the subjects underwent a thorough ophthalmic evaluation, including slit-lamp biomicroscopy, IOP measurement (Goldmann applanation tonometry), gonioscopy, fundus examination, ultrasound biomicroscopy (UBM), and B-scanning. All participants underwent VF testing, using Octopus 101 (Haag-Streit, Inc., Bern, Switzerland, Dynamic Strategy). The patients enrolled satisfied the following criteria: (1) presenting an IOP > 21 mmHg (Goldmann applanation tonometry); (2) with glaucomatous optic nerve damage and matching VF defects of Octopus perimetry. (3) the presence of an occluded angle > 90° in the affected eye, verified by gonioscopy. All eyes underwent gonioscope examination to confirm the existence of a narrow-angle pupillary block component. These CPACG with inadequate control of IOP with maximal and tolerated medical treatment and still had progressive VF loss, would undergo trabeculectomy surgery.

Serving as the non-high IOP control, the ARC controls were free of glaucoma based on IOP < 21 mmHg with healthy appearance of the optic nerve. These patients underwent routine phacoemulsification surgery with a foldable intraocular lens implant.

The patients were excluded from the study with any of the following conditions: individuals with systemic, autoimmune, inflammatory, and immunosuppressive diseases and any past history of ocular surgery or preexisting ocular disease, such as retinal artery disease, retinal vein occlusions, and retinal vascular diseases including diabetic retinopathy and age-related macular degeneration. In addition, the patients with any of the following clinical features were also excluded by a detailed clinical ophthalmic evaluation: secondary angle closure due to trauma, uveitis lens subluxation, iris neovascularization, trauma, and intumescent lens. Moreover, the eyes with acute angle closure glaucoma and treatment with laser iridotomy within 6 weeks of ocular surgery were also excluded.

A total of 51 eyes fit for the criteria above were enrolled in the study, including CPACG patients (n = 22) and ARC controls (n = 29).

2.2. Sample collection

Preoperative aqueous humor was obtained from the anterior chamber as follows: Briefly, aqueous humor samples (100 μl) were obtained at the beginning of surgery for CPACG or ARC through a limbal paracentesis using a 0.1-ml syringe. All samples collected were frozen immediately and stored at −80 °C until analysis. The levels of IL-36, IL-37 and IL-38 were measured, using ELISA kits (Shanghai Ding Biological Technology Co., LTD, China). The experimental procedure was conducted according to the instructions from the manufacturer. All measurements were performed in triplicate.

2.3. Statistical analysis

The data was expressed in the form of mean ± SD and analyzed via statistical software (GraphPad InStat). After comparing the ARC and the CPACG group, Mann-Whitney *U* test and Fisher's exact probability test were performed to respectively evaluate the differences in continuous data including age, IOP, number of glaucoma medications, cytokine concentrations, and those in categorical data including sex. The GraphPad Prism 6 software (GraphPad, La Jolla, CA, USA) was used to generate the Scatter plots for cytokines. For comparisons of the inflammatory cytokines between the ARC and the CPACG group, $p < 0.05$ was considered to be statistically significant based on the Mann-Whitney *U* test. In addition, three cytokines levels were determined simultaneously and results with $p < 0.05$ were considered significant for single-cytokine measurements. Associations between these cytokines with ophthalmologic measurements were described by Pearson's correlation coefficients.

3. Results

3.1. Patients demographic data

The demographic and clinical data of the subjects, including age, sex, number, and details of glaucoma medication treatment are presented in Table 1. There was no significant difference in average age between the CPACG patients and ARC patients (64.96 vs. 71.72 years, $p > 0.05$). The mean IOP in the eyes of CPACG patients was higher than that in ARC (22.80 ± 7.01 vs. 15.67 ± 2.35 mmHg, $p = 0.003$). The MDVF in the eyes of CPACG patients was significantly higher than that in ARC (19.66 ± 4.77 vs. 0.5 ± 0.2 mmHg, $p < 0.001$).

3.2. The comparison of cytokines between CPACG and ARC

The aqueous humor concentration of IL-36, IL-37 and IL-38 were measured in both CPACG and ARC groups (Table 2). Compared to ARC group, CPACG group showed a significantly higher proportion of samples of the three cytokines:IL-36 (9.23 ± 0.7205 vs. 7.392 ± 0.4883 pg/ml, $p < 0.05$), IL-37 (28.5 ± 1.832 vs. 23.35 ± 1.658 pg/ml, $p < 0.05$), and IL-38 (23.65 ± 1.609 vs. 19.41 ± 1.354 pg/ml, $p < 0.05$) (Fig. 1).

Table 1
Demographic and ophthalmologic measurements details of CPACG and ARC.

Characteristics	ARC (n = 29)	CPACG (n = 22)	p-Value
Age (years)			
Mean ± SD	71.72 ± 9.24	67.36 ± 10.47	0.729
Range	51–88	50–83	
Sex			
Men	14	15	
Women	15	7	
IOP (mmHg)			
Mean ± SD	15.67 ± 2.35	22.80 ± 7.01	0.0003
Range	9.1–20	10–37	
Glaucoma medications, n			
α-Agonists eye drop	0	15	
β-Blockers eye drop	0	19	
Carbonic anhydrase inhibitors eye drop	0	10	
Prostaglandin analogs eye drop	0	4	
MDVF (dB)			
Mean ± SD	0.5 ± 0.2	19.66 ± 4.77	< 0.001
Range	0–1	11.2–29	

Data are expressed as the mean ± SD; SD: Standard deviation. $p < 0.05$ is considered significant.

Table 2
Aqueous humor concentrations (pg/ml) of Cytokines (IL-36, IL-37 and IL-38) in ARC and CPACG patients.

Cytokine	ARC(n = 29)		CPACG(n = 22)		p-Value*
	Mean ± SD	Range	Mean ± SD	Range	
IL-36	7.392 ± 0.4883	3.93–10.1	9.23 ± 0.7205	5.51–12.55	< 0.05
IL-37	23.35 ± 1.658	11.56–38.08	28.5 ± 1.832	13.54–43.35	< 0.05
IL-38	19.41 ± 1.354	8.55–27.03	23.65 ± 1.609	12.17–34.73	< 0.05

Data is expressed as the mean ± SD; SD: Standard deviation. $p < 0.05$ is considered significant.

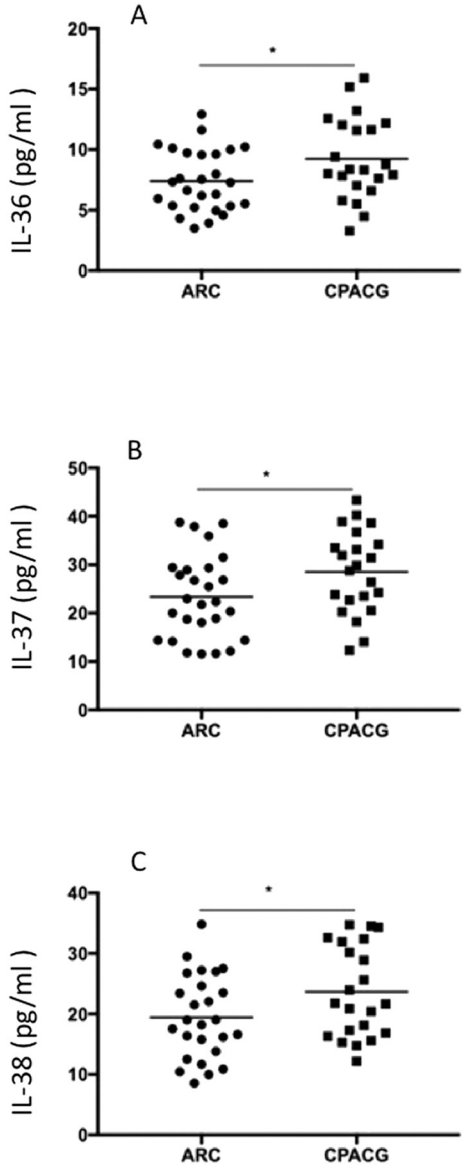

Fig. 1. Scatter plots of these three cytokines (IL-36, IL-37, and IL-38) in CPACG and ARC eyes. (A, B and C). The solid line marks the median concentrations. (*p < 0.05).

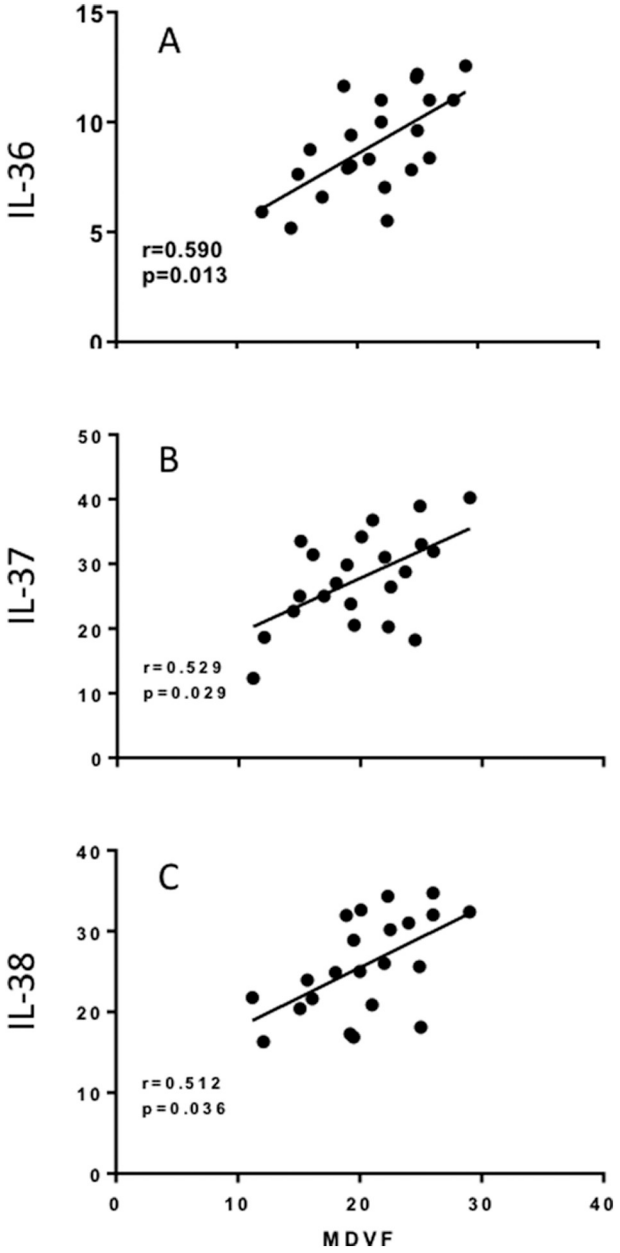

Fig. 2. The correlation between MDVF and IL-36 (r = 0.590, p = 0013), IL-37 (r = 0.529, p = 0.029), IL-38 (r = 0.512, p = 0.036).

3.3. Correlation analysis

The correlations between each cytokine and age and MDVF are shown in Table 2. No correlation was found between the aqueous cytokine levels and age. However, MDVF was found to correlate positively with IL-36 (r = 0.590, p = 0.013), IL-37 (r = 0.529, p = 0.029), IL-38 (r = 0.512, p = 0.036) (Fig. 2).

4. Discussion

In the current study, there was significantly elevated IL-36, IL-37 and IL-38 in the aqueous humor from the CPACG group than that from ARC group. In addition, IL-36, IL-37 or IL-38 correlated with MDVF in CPACG patients. Aqueous humor is the product of the ciliary body

which is able to reflect the intraocular environment, and some cytokines levels in the aqueous humor have been changed in many ocular diseases, including glaucoma [3,12–15]. It is becoming one of the hot topics to explore whether there is modification of pro and anti-inflammatory cytokines in the aqueous humor in ophthalmology in recent years. It is interesting to find that there is involvement of IL-36, IL-37 and IL-38 in the pathogenesis of CPACG, which is supported by the findings that cytokines in the aqueous humor have some connections with the pathophysiology of glaucoma, and even affect the prognosis of glaucoma [16–18].

IL-36 and its receptors, expressed in many organs/tissues, including skin, lung and gut, participate in inflammatory responses [19]. Upregulated IL-36 is observed on epithelial cells and specific immune cells, inducing further cellular activation and secretion of cytokines and chemokines, leading to recruitment and activation of a variety of immune cells [20]. It is detected highly elevated in the aqueous humor of acute uveitis, involved with autoreactive T cells immune response [7], suggesting the involvement of IL-36 in the development of host immunity against pathogenic invasion. This is partially in line with our observation that elevated IL-36 in the aqueous humor from CPACG. Although there is non-infectious cause(s) involving in the development of CPACG, our data suggest that the pathogenesis of CPACG is related to host immunological mediated inflammation in the microenvironment, which is consistent with the most recent publication, showing that T cell mediated immunological response contributes to the development of glaucoma [6]. Furthermore, we found that there was a significant correlation between IL-36 and MDVF, which is further supporting the possible contribution of IL-36 to the progression of glaucoma.

IL-37 is an anti-inflammatory cytokine and upregulation of IL-37 is observed in many inflammatory diseases, e.g. synoviocytes of patients with rheumatoid arthritis [21], foam cells of atherosclerosis monocytes from patients with lupus [22]. Thus, IL-37 plays a crucial role in regulating the inflammatory response. We observed increased IL-37 in CPACG, which is in line with such anti-inflammatory property in nature, suggesting that CPACG is a non-infectious inflammation. Moreover, a significant correlation between IL-37 and MDVF in our current study is another objective evidence supporting the immunological mediated pathogenesis of glaucoma.

Finally, IL-38, also an anti-inflammatory cytokine, is reported in the aqueous humor from AAU patients [8,23]. We found increased IL-38 in aqueous humor of CPACG, compared to that of ARC. Our explanation is that increased anti-inflammatory cytokine, IL-37 and IL-38, may be a compensatory mechanism in the aqueous humor micro-environment in CPACG. However the effect of IL-37 and IL-38 may be compromised due to an unknown underlying pathway, which will be determined in our future experiment. Similarly there was a significant correlation between IL-38 and MDVF in our current study, which further supports our hypothesis that there is immunological mediated pathogenesis of glaucoma.

It has been reported that the release of inflammatory cytokines from damaged RGCs results in MDVF [2,17,24], which is in line with our current finding that there was a correlation between proinflammatory cytokines (IL-36) as well as anti-inflammatory cytokines (IL-37 and IL-38), and MDVF.

It is well known that the eye is an immunologically “privilege site”, although it has been demonstrated that there are a number of immunological diseases that involve the eye [25,26]. Thus, although there is ~20% elevation of IL-36, 37 and 38 in CPACG, compared to ARC, such an induction is likely to have a substantial impact within the eye, compared to comparable systemic levels, e.g. in the serum or plasma. Such an explanation is supported by our findings, showing that there are significant correlations between these three individual cytokines and disease score.

There are some potential limitations in the present study. Restricted by the traditional Elisa, it is difficult to measure various cytokines using limited aqueous humor, thus we only measured the general expression of IL-36 without testing the expression quantity of each subtype. Another limitation of the study was its sample size, as the number of patients enrolled was relatively low. Nevertheless, the results were statistically significant, so the relatively small number of patients may serve to strengthen the results and conclusions of the study.

In conclusion, IL-36, IL-37 and IL-38 related to inflammation showed elevated levels in the aqueous humor of eyes in CPACG compared to ARC. Up-regulated IL-36, IL-37 and IL-38 showed a positive correlation with MDVF, even in the absence of clinically observable inflammation, suggesting a plausible role for inflammatory cytokines in the pathogenesis of CPACG. However, further studies on targeting these cytokines will be helpful to elucidate their underlying mechanism in angle closure glaucoma.

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Conflicts of interest

The authors declare that they have no conflict of interest.

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