Frontiers in Bioscience Landmark

Front. Biosci. (Landmark Ed) **2024**; 29(1): 29
https://doi.org/10.31083/j.fbl2901029

Original Research

Cytokine Profiling in Aqueous Humor of Glaucoma Patients and in Retinas from an *Ex Vivo* Glaucoma Animal Model

Jiancen Tang[1,†], Mengsha Sun[1,†], Yuan Feng[2], Verena Prokosch[2,*], Hongping Cui[1,*], Hanhan Liu[2,*]

[1]Department of Ophthalmology, Shanghai East Hospital, Tongji University School of Medicine, 200120 Shanghai, China
[2]Department of Ophthalmology, Faculty of Medicine and University Hospital of Cologne, University of Cologne, 50937 Cologne, Germany
*Correspondence: verena.prokosch@uk-koeln.de (Verena Prokosch); hpcui@vip.163.com (Hongping Cui); hanhan.liu@uk-koeln.de (Hanhan Liu)
[†]These authors contributed equally.

Academic Editor: Adrian Gericke
Submitted: 17 March 2023 Revised: 26 October 2023 Accepted: 1 November 2023 Published: 19 January 2024

Abstract

Background: Although the current role of cytokines and neuroinflammation in glaucoma remains obscure, it represents an expanding field in research. The purpose of this study was to analyze cytokines in the aqueous humor (AH) of glaucoma patients and in retinas from an *ex vivo* glaucoma animal model, to aid in determining the role of neuroinflammation in glaucoma. **Methods**: AH samples were collected from 20 patients during cataract surgeries (controls: n = 10, age = 70.3 $\pm$ 9.742; glaucoma: n = 10, age: 66.5 $\pm$ 8.073) in Shanghai East Hospital, an affiliate of Tongji University, between September 2018 and March 2019 and analyzed in duplicate by Luminex cytokine polystyrene color bead-based multiplex assay. Retinas from female Sprague-Dawley rats (n = 6) were harvested *ex vivo* and cultured with or without 60 mmHg of hydrostatic pressure for 24 hours. Retinal ganglion cells (RGCs) were quantified using Brn3a staining. Cytokines in the retina and culture medium were analyzed by rat cytokine array (Abcam). **Results**: At baseline, patients with primary angle closure glaucoma (PACG) have significantly lower levels of IL-6 and IP-10 and a higher level of PDGF-BB in their AH, compared to the controls. Postoperatively, patients with PACG have significantly higher levels of IL-1ra, IL-13, and MIP-1α and a lower level of IL-6. Elevated hydrostatic pressure led to significant RGC loss in the retina, *ex vivo*, as well as the upregulation of ciliary neurotrophic factor (CNTF), IL-6, IL-10, IL-4, and TIMP-1 alongside the downregulation of PDGF-AA, MMP-8, TNF-α, and IFN-γ. Furthermore, eight cytokines were detected as being downregulated in the culture medium, including PDGF-AA, MMP-8, and IL-4. **Conclusions**: Proinflammatory cytokines showed changes in both AH and *ex vivo*. Further studies are needed on the role of these cytokines and their corresponding signaling pathways in both neurodegeneration and glaucoma.

Keywords: glaucoma; neurodegeneration; cytokine; inflammation; retina culture; retinal ganglion cell

1. Introduction

Glaucoma is a group of optic neuropathies characterized by progressive loss of retinal ganglion cells and their axons and represents a leading cause of blindness. Glaucoma can be classified into two broad categories: open-angle glaucoma and angle-closure glaucoma [1]. Elevated intraocular pressure (IOP) is the main risk factor in both types of glaucoma, however, adequate IOP reduction cannot halt the progress of the disease in most patients. Additionally, the pathophysiology remains obscure [2].

Increasing evidence suspects neuroinflammation, an innate immunological response of the nervous system that comprises microglia, astrocytes, cytokines, and chemokines, as a key process in neurodegenerative disease [3–7]. High IOP is a potential stimulus associated with the production of proinflammatory cytokines, such as chemokine (C-C motif) ligand 2 (CCL2), CCL1, CCL1, Interleukin-10 (IL-10), interleukin-6 (IL-6), and activated transforming growth factor-beta 2 (TGF-β2) [8]. Increased levels of TGF-β2 and tumor necrosis factor-α (TNF-α), and IL-6, interleukin-8 (IL-8), interleukin-9 (IL-9), interleukin-10 (IL-10), interleukin-12 (IL-12), interferon-γ (IFN-γ), interferon-α (IFN-α), vascular endothelial growth factor (VEGF), and monocyte chemoattractant protein-1 (MCP-1), alongside the chemokine (C-X-C motif) ligand 9 (CXCL9) have even been found in the aqueous humor (AH) of glaucoma patients [9–14]. It is indicated that these cytokines are produced locally by ocular tissues [15].

Although the exact source of the inflammatory cytokines and their roles in neuroinflammation is unclear, the cytokine levels in AH have been reported to be associated with the clinical prognosis of retinal disorders [16–19]. However, it is difficult to verify these results and to study the molecular mechanisms of the findings in relation to glaucoma in human ocular tissues since human ocular material can only be obtained postmortem and human patients usually have other onset systematic diseases. Thus, the experimental glaucoma models provide the possibility to investigate the precise molecular and cellular changes in a standardized manner. Most of the progress in the research of glaucoma has been based on experimentally inducible

glaucoma animal models [20–24]. *Ex vivo* retina models are common research tools that provide the additional positive effect of improved experimental standardization and a reduction in the number of animals that are used in the research [25,26].

The purpose of this study was to first analyze the cytokines in the AH of patients with primary angle closure glaucoma (PACG). Secondly, to analyze the cytokines in the retinas from an *ex vivo* glaucoma model. The largely unspecified roles of neuroinflammation in glaucoma prompted us to further compare the cytokine profiles between glaucoma patients and in the *ex vivo* animal model, as well as their corresponding signaling pathways. Better insight into the intraocular condition in glaucoma and the glaucoma model is fundamental for optimizing future research in both clinical and basic science.

2. Materials and Methods

2.1 Patient Recruitment

A total of 20 patients, including seven males (14 eyes) and thirteen females (26 eyes), underwent cataract surgery from September 2018 to March 2019 in the ophthalmology department at Shanghai East Hospital and were included in this study. Participants were adults with PACG and their age-matched controls had age-related cataracts (10 patients/group). PACG was defined using the criteria from the International Society for Geographical and Epidemiological Ophthalmology (ISGEO) [27]. Briefly, PACG diagnosis was defined by a primary anatomic narrow-angle (180 ° or more of iridotrabecular contact assessed by indentation gonioscopy and ultrasound biomicroscopy (SW-3200L, SUOER, Tianjin, China), with glaucomatous optic neuropathy (a vertical cup/disc [C/D] ratio $>$0.7 and/or C/D asymmetry $>$0.2 and/or focal notching of the neuroretinal rim), and compatible visual field defects measured using the Octopus 900 perimeter (Haag-Streit Inc., Kniz, Switzerland). Patients with histories of acute glaucoma attack, ocular surgery, an advanced visual field defect, and uncontrolled IOP were excluded. All the PACG patients enrolled in the present study had controlled IOP with only pilocarpine eyedrops (assessed by 24 h IOP profile). Exclusion criteria included previous trauma, fundus pathology, retinal diseases, other autoimmune disease, and a history of previous ocular surgery. This prospective study was approved by the research ethics boards at Shanghai East Hospital of Tongji University (EC. D (BG) .016.03.1-2018-007), and adhered to the tenets of the Declaration of Helsinki. All participants provided written informed consent prior to participation in the study.

2.2 Aqueous Humor Collection

A total of 40 aqueous humor samples were collected at the outset of cataract surgeries from both eyes of 20 patients. The cataract surgery and the aspiration of the AH were carried out in sterile conditions by the same surgeon and under a surgical microscope for ophthalmology (Leica M844 F40 / F20, Wetzlar, Germany). The cytokines from the AH of the first eye, before the first eye surgery, were considered the baseline, while those from the second eye after the first eye surgery were defined as postoperation.

After topical anesthesia with Alcaine solution (0.5% proparacaine hydrochloride ophthalmic solution, S.A. Alcon-Couvreur N.V. Puurs, Belgium), a clear corneal incision was made at “2 o’clock” using a 15-degree angle blade (Model: 8065921501, Alcon, Geneva, Switzerland). Through the cornea incision, approximately 150 µL of the AH was aspirated slowly using a 1 mL syringe attached to a 30-gauge needle, on a plane approximately parallel to the long axis of the elliptical lens. Each AH sample was centrifuged, and the supernatant was subsequently transferred into a sterile Eppendorf tube, snap-frozen in liquid nitrogen, and stored at –80 °C.

2.3 Cytokine Analyses

Twenty-seven cytokines, chemokines, and growth factors were measured in the AH samples using Luminex cytokine polystyrene color bead-based multiplex assay, in duplicate.

The measured cytokines, chemokines, and growth factors were as follows: Interleukin-10 (IL-10), interleukin-13 (IL-13), interleukin-12p70 (IL-12p70), interleukin-15 (IL-15), interleukin-17 (IL-17), interleukin-1rα (IL-1rα), interleukin-1β (IL-1β), interleukin-2 (IL-2), interleukin-4 (IL-4), interleukin-5 (IL-5), interleukin-6 (IL-6), interleukin-7 (IL-7), interleukin-8 (IL-8), interleukin-9 (IL-9), granulocyte-colony stimulating factor (G-CSF), granulocyte–macrophage colony-stimulating factor (GM-CSF), interferon-γ (IFN-γ), tumor necrosis factor-α (TNF-α), eotaxin (CCL11), interferon gamma-induced protein 10 (IP-10), monocyte chemoattractant protein-1 (MCP-1), macrophage inflammatory protein-1α (MIP-1α), macrophage inflammatory protein-1β (MIP-1β), regulated upon activation, normal T cell expressed and presumably secreted (RANTES), basic fibroblast growth factor (bFGF), platelet-derived growth factor (PDGF-BB), and vascular endothelial growth factor (VEGF).

2.4 Ex Vivo Glaucoma Model

Age-matched female Sprague-Dawley rats (n = 12, 250–300 g) were used in this study. All animals were housed in standard accommodation provided by the Animal Institute of the Faculty of Medicine of the University of Cologne. Food and water were provided ad libitum on a 12-hour diurnal cycle.

All experimental procedures complied with the German animal welfare law, which is in line with the European Community law, and the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research. The animal protocols used in this study were reviewed and approved by the governmental body responsible for ani-

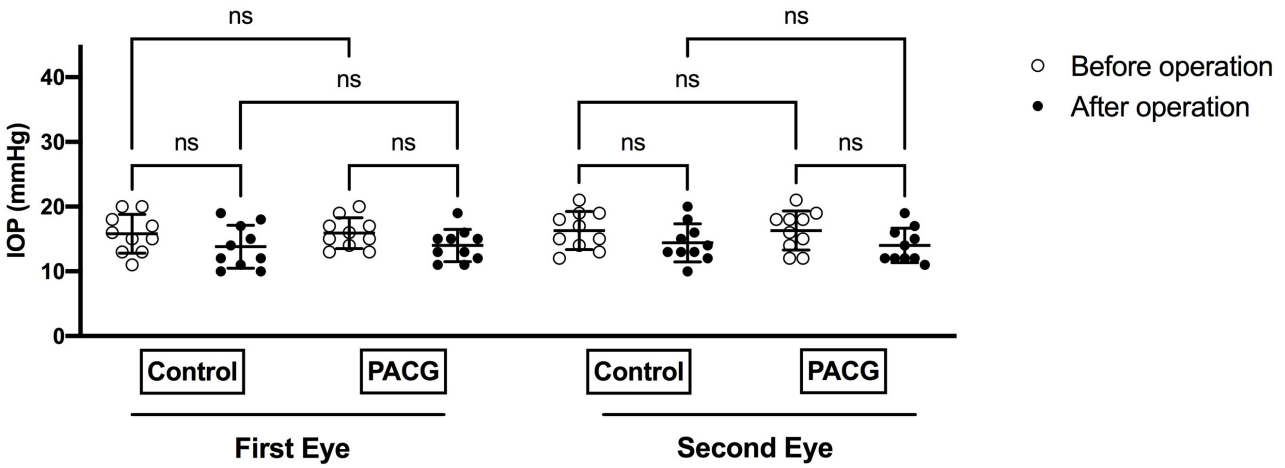


Fig. 1. IOP between PACG and control groups before and after the cataract operation. PACG patients had their IOP medically controlled throughout the study using only pilocarpine in the normal range. There is no significant difference in IOP between groups before or after the cataract operation. n = 10. IOP, intraocular pressure; ns, not significant. PACG, primary angle closure glaucoma.

mal welfare in the state of North Rhine-Westphalia, Germany (Paragraph 4.21.003, Landesamt für Natur, Umwelt und Verbraucherschutz Nordrhein-Westfalen). Sprague-Dawley rats were sacrificed under a CO_2 atmosphere. The eyes were removed immediately and placed in phosphate-buffered saline (PBS, pH 7.4). The anterior segment of the eye was removed under the microscope and each whole retina was carefully explanted. The retina was laid flat on Millipore filters (Millipore, Millicell, Cork, Ireland) with the retina ganglion cell side facing up. The vitreous body was subsequently removed using forceps.

The retinal explants were transferred to a Lumox dish 50 (Sarstedt, Nümbrecht, Germany). Every two retinal explants were assigned to a dish and were cultured in a 5 mL culture medium. The culture medium was Dulbecco's Modified Eagle's Medium/Nutrient Mixture F-12 (DMEM/F12; Gibco BRL, Eggenstein, Germany) with an additional 10% FCS and 1% penicillin/streptomycin. The control groups and the IOP groups were placed in the same pressure incubation chamber. The chambers were put in an incubator (Heracell 240i, Thermo Scientific, Niederelbert, Germany) at 37 °C in a 5% CO_2 humidified atmosphere for 24 hours. The control group was cultured without additional pressure, although the IOP group was cultured under fluctuating pressure for the first 6 hours (pressure in the chamber was increased to 60 mmHg, then reduced gradually every 15 minutes until 0 mmHg), and the other 18 hours were without pressure.

2.5 Quantification of Retinal Ganglion Cells

Retinal ganglion cells (RGCs) were determined by immunohistochemical staining against the brain-specific homeobox/POU domain protein 3A (Brn3a, sc-8429, Santa Cruz Biotechnology, Dallas, TX, USA) using retinal flat-mounts. One-quarter of each retinal sample was carefully separated under a microscope. After washing in PBS, the retinal tissue was fixed in 4% formalin solution for 30 minutes (Carl Roth, Karlsruhe, Germany) at room temperature and in 30% sucrose solution overnight. The retinas were permeabilized with PBS-T (0.3% Triton x-100 in PBS) for 30 minutes. Subsequently, the tissues were blocked with Blotto solution (1% milk powder and 0.3% Triton x-100 in PBS) for 30 minutes at room temperature and incubated with the primary antibody (1:200; mouse Anti-Brn3a monoclonal antibody, EMD Millipore, Darmstadt, Germany) diluted in Blotto solution at 4 °C overnight. After thorough washing with PBS-T, the retinal samples were incubated with the secondary antibody (1:1000; goat anti-mouse IgG Alexa Fluor™ 488, Abcam, Cambridge, UK) for 1 hour at room temperature. The retinal samples were flat mounted on the objective, the RGCs were visualized using a fluorescent microscope (Zeiss Axio Imager.M2, Carl Zeiss Microscopy Deutschland GmbH, Oberkochen, Germany) at 20× magnification. Photos were taken at the same six positions for each retinal quarter. The number of Brn3a-positive cells was counted using ImageJ (mageJ Fiji v_1, https://imagej.nih.gov/ij/).

2.6 Rat Cytokine Antibody Array in Retinas and Culture Medium

The rest of the rat retinas from 12 eyeballs were lysed by T-PER tissue protein extraction reagent. After protein quantification by BCA assay, retinal protein samples from both groups were pooled equally into three biological replicates, represented by R1, R2, and R3 (N = 3 replicates per group), the culture medium was pooled equally into three biological replicates per group. Total protein samples of 250 µg per biological replicate were added to 1 mL of 1X Blocking Buffer (final volume) and subsequently subjected to the array membrane of the rat cytokine antibody array (ab133992, Abcam, Cambridge, UK), the procedure was carried out following the manufacturer's manual.

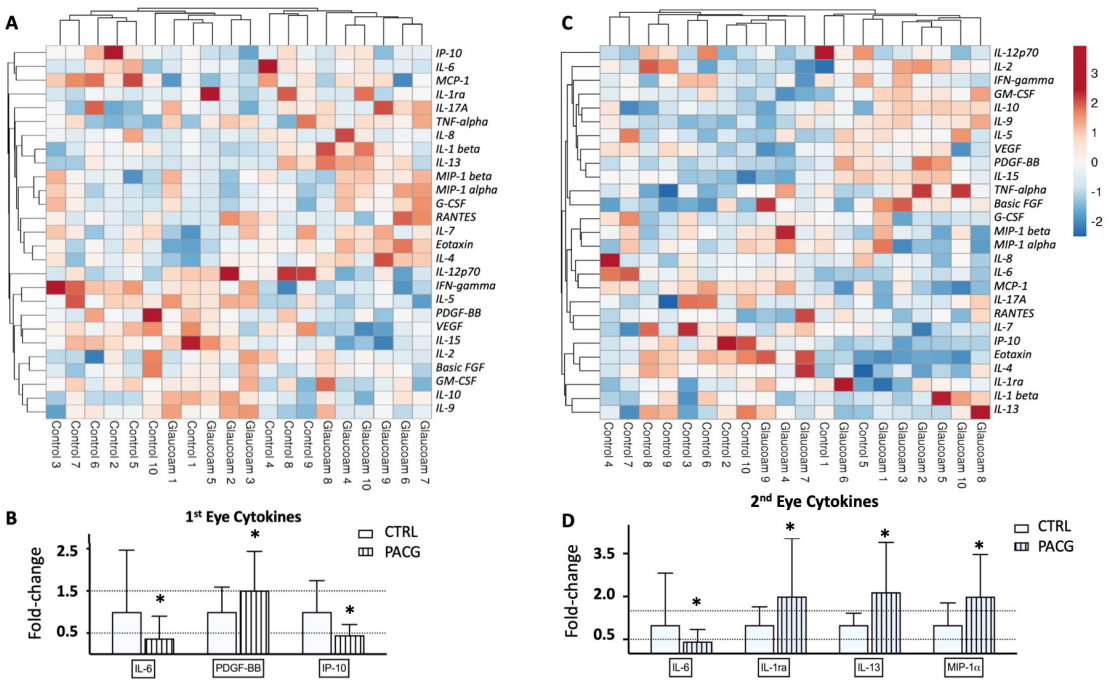


Fig. 2. Aqueous humor cytokine profiles for PACG and control patients at baseline and postoperation. (A) Heat map depicts the baseline hierarchical clustering of the differentially expressed cytokines in the PACG group compared to the control group. The relative abundance of the cytokines is represented as heat maps for each cluster. (B) Differentially expressed cytokines in PACG and control patients at baseline. (C) Heat map depicts the postoperative hierarchical clustering of the differentially expressed cytokines in the PACG group compared to the control group. (D) Differentially expressed cytokines in the control and PACG groups postoperatively. n = 10, $^{*}p < 0.05$. CTRL: control; PACG, primary angle closure glaucoma.

2.7 Bioinformatics, Functional Annotation, Pathways Analyses, and Statistics

Unsupervised hierarchical clustering analysis was performed using the z-scores of the cytokine concentrations following Euclidean distance (linkage = average; preprocess with k-means) and illustrated in a heat map. The significant differentially expressed cytokines, chemokines, and growth factors (fold change cutoff of $\geq$1.5 or $<$2/3) in both PACG patients and the glaucoma animal model were further subjected to the Search Tool for the Retrieval of Interacting Genes/Proteins (STRING) for protein–protein association networks [28] and pathways analysis. Statistical analysis and presentation were performed by GraphPad Prism (version 9.2, Mac OS X, GraphPad Software, San Diego, CA, USA, www.graphpad.com). Data values are presented as mean $\pm$ SD, Mann–Whitney tests were used to compare the age and operation interval of the patients, while an unpaired *t*-test was used to compare the RGC numbers between groups. A value of $p < 0.05$ was considered statistically significant.

3. Results

3.1 Patient Demographics

In this study, seven male (14 eyes) and thirteen female (26 eyes) patients were recruited and distributed into two groups: patients diagnosed with PACG (PACG, n = 10) and a control group of cataract participants (CTRL, n = 10, Table 1). There was no significant difference in age, gender, and the interval and operative times between the groups. All PACG patients had their IOP medically controlled with only pilocarpine eyedrops without prior glaucoma surgery (Fig. 1). All patients recovered well from the phacoemulsification without intraoperative and postoperative complications.

3.2 Cytokine Profiling of AH from the Controls and Patients with PACG at Baseline and Postoperation

Luminex cytokine polystyrene color bead-based multiplex assay was used to quantify and distinguish AH cytokine profiles acquired from patients during their first and second cataract surgeries. The AH acquired from the first operation was assumed as the baseline and those acquired from the second operation were set as postoperative.

There is a subtle separation in cytokine profile between the PACG vs. controls (Fig. 2A). At baseline, 3 of the 27 cytokines were found to be significantly differentially expressed in the AH samples of glaucoma patients compared to the control group (Fig. 2B). Patients with PACG has significantly lower levels of IL-6 (FC = 0.37) and IP-10 (FC = 0.447) and a significantly higher level of PDGF-BB (FC = 1.508) in their AH, compared to the controls.

Table 1. Patient demographics.

Demographic data	PACG group	CTRL group	*p*-value
Age (years)	66.5 ± 8.07	70.3 ± 9.74	0.355
Male	3 (30%)	4 (40%)	0.439
Female	7 (70%)	6 (60%)	
Interval between operations (days)	12.9 ± 8.02	10.9 ± 6.28	0.543
Operation time (minutes)	12.95 ± 1.83	12.7 ± 2.76	0.341

Data are represented as mean ± SD (n = 10) or patient number (%).
CTRL, control; PACG, primary angle closure glaucoma.

Table 2. List of most significantly modulated KEGG pathways in the PACG baseline and postoperative groups compared to the control.

PACG baseline			
KEGG pathways	Genes	Strength	FDR
Cytosolic DNA-sensing pathway	*IL-6*, *IP-10*	2.32	0.0143
IL-17 signaling pathway	*IL-6*, *IP-10*	2.15	0.0155
Viral protein interaction with cytokine and cytokine receptor	*IL-6*, *IP-10*	2.13	0.0155
Toll-like receptor signaling pathway	*IL-6*, *IP-10*	2.11	0.0155
TNF signaling pathway	*IL-6*, *IP-10*	2.07	0.0155
Cytokine-cytokine receptor interaction	*IL-6*, *IP-10*	1.67	0.0354
PACG postoperative			
KEGG pathways	Genes	Strength	FDR
Inflammatory bowel disease	*IL-6*, *IL-13*	2.21	0.0134
IL-17 signaling pathway	*IL-6*, *IL-13*	2.03	0.0206
Viral protein interaction with cytokine and cytokine receptor	*IL-6*, *MIP-1α*	2.01	0.0206
Cytokine-cytokine receptor interaction	*IL-6*, *IL-13*, *IL-1ra*, *MIP-1α*	1.84	2.03×10^{-5}
JAK-STAT signaling pathway	*IL-6*, *IL-13*	1.79	0.0367

FDR, false discovery rate; KEGG, Kyoto Encyclopedia of Genes and Genomes.

In the second eye, there was a clearer separation in cytokine profile between the PACG and control groups (Fig. 2C). Four cytokines were found to be significantly differentially expressed in the AH samples between the PACG and the control groups (Fig. 2D). Patients with PACG had significantly higher levels of IL-1ra (FC = 2.0), IL-13 (FC = 2.152), and MIP-1α (FC = 2.0) and a significantly lower level of IL-6 (FC = 0.425) in their AH, compared to the controls.

3.3 Significant RGC Loss Ex Vivo in the Glaucoma Animal Model

Culturing under fluctuating hydrostatic pressure for 24 h resulted in the number of RGCs in the retinal samples being significantly reduced (167.52 ± 39.52 RGC/field) compared to the control (205.59 ± 28.38 RGC/field, Fig. 3, $^{****}p < 0.0001$, n = 6, mean ± SD).

3.4 Cytokine Profiling of Retinal Samples from the Glaucoma Animal Model and Controls

A heat map with unsupervised hierarchical clustering of retinal cytokines in the glaucoma model and the control group shows a distinct difference in the cytokine makeups between groups (Fig. 4A). Among 34 cytokines, 8 cytokines were significantly upregulated in the glaucoma model, while 7 cytokines were significantly downregulated in the glaucoma model (fold change cutoff of >1.5 or <2/3, Fig. 4B). The three most significantly upregulated cytokines were IL-6, IL-10, and the ciliary neurotrophic factor (CNTF). The three most significantly downregulated cytokines were the receptor for advanced glycation endproducts (RAGE), platelet-derived growth factor AA (PDGF-AA), and prolactin receptor (prolactin R).

3.5 Cytokine Array of Culture Medium from Glaucoma Animal Model and Controls

The cytokine array was carried out in a pooled culture medium from the glaucoma model and the controls (n = 3). Eight cytokines were downregulated in the culture medium of the glaucoma model compared to the control (fold change cutoff of <2/3, $p < 0.05$, Fig. 5), the three most significantly downregulated cytokines were PDGF-AA, matrix metalloproteinase-8 (MMP-8), and IL-4.

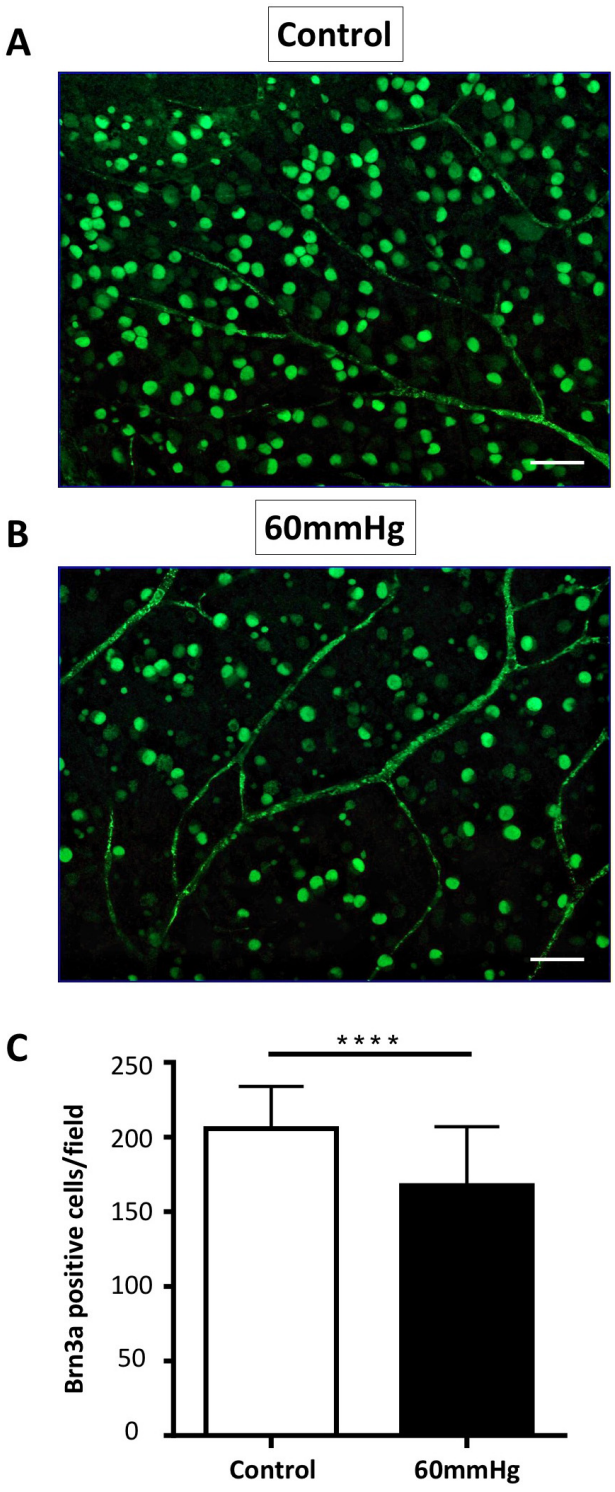


Fig. 3. Significant RGC loss *ex vivo* in the glaucoma animal model. (A,B) Representative fluorescence microscopy of Brn3a positive RGCs in retinal explants after culturing 24 h with or without fluctuating pressure. (C) Compared to the control (205.59 ± 28.38 RGC/field), culturing under fluctuating pressure resulted in significant RGC loss in retinal explants (167.52 ± 39.52 RGC/field). n = 6, $^{****}p < 0.0001$. RGCs, retinal ganglion cells. Scale bar = 50 µm.

3.6 Functional Enrichment Analysis of Significantly Altered Cytokines in PACG Patients and Glaucoma Animal Model

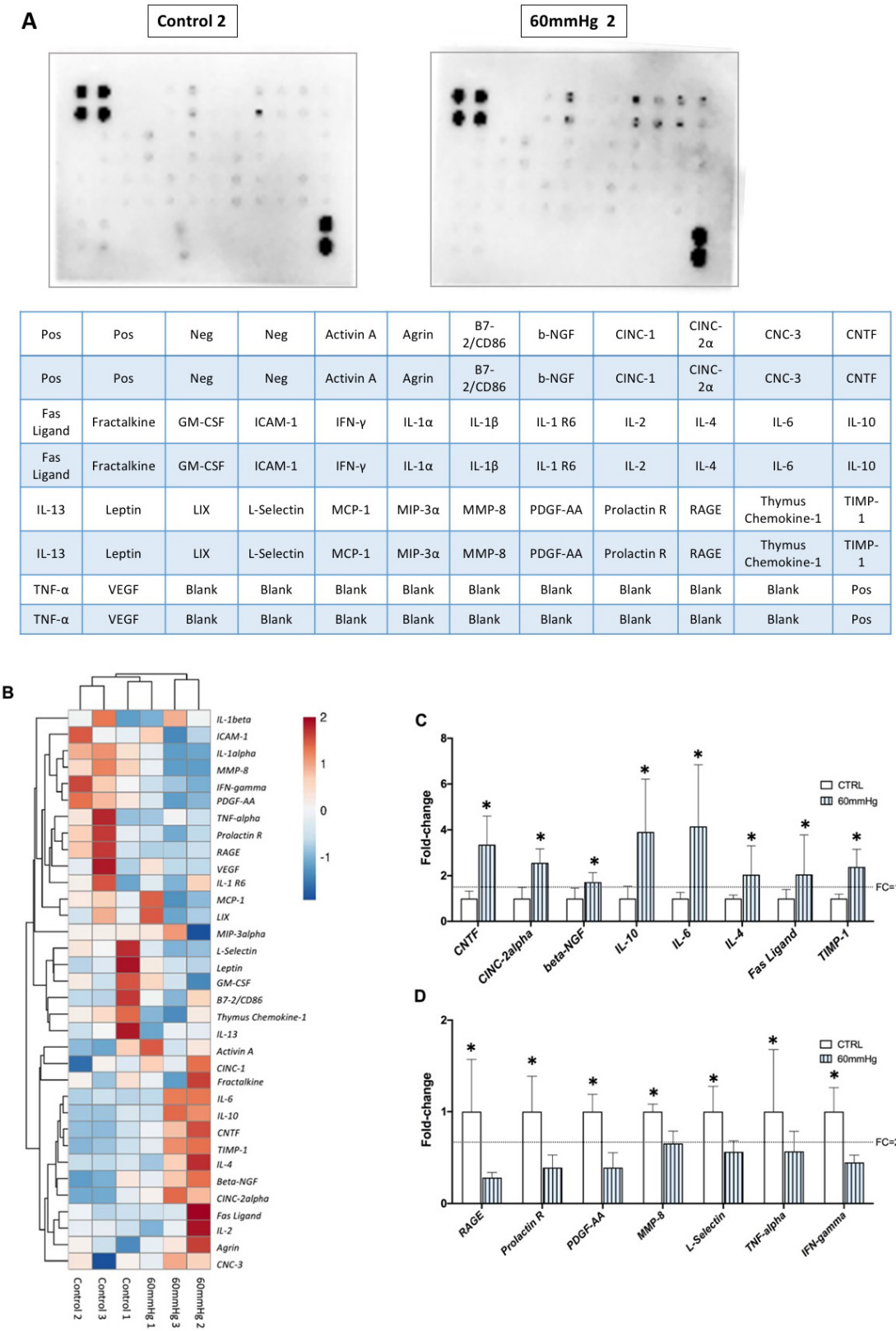
The differentially expressed cytokines from both the patients and the glaucoma animal model were respectively entered into Search Tool for the Retrieval of Interacting Genes/Proteins (STRING) for protein–protein association networks (Fig. 6 and Table 2) [28]. Using STRING analysis (Version 11, https://string-db.org/), pathways such as the IL-17 signaling pathway, viral protein interaction with cytokine, and cytokine receptor and cytokine–cytokine receptor interaction were shown to be deregulated in all groups. Whereas pathways such as the TNF signaling pathway and toll-like receptor signaling pathway were altered in the PACG baseline group and glaucoma model, while the inflammatory bowel disease signaling pathway and JAK/STAT signaling pathway were altered in the PACG postoperative group and glaucoma model. Nonetheless, there are pathways that were exclusively deregulated in the glaucoma animal model, such as the MAPK, PI3K–Akt, HIF-1, neurotrophin, and apoptosis signaling pathways.

4. Discussion

The aim of this study was to first profile the cytokines in the AH of patients diagnosed with PACG in comparison to non-PACG patients at both the baseline and after cataract surgery. Luminex cytokine polystyrene color bead-based multiplex assay was used to quantify and distinguish 27 cytokines simultaneously. Our results showed a differential expression of AH cytokines in PACG patients both at baseline and postoperatively. At baseline, an increased abundance of PDGF-BB was observed, while IL-6 and IP-10 were decreased. After cataract surgery, IL-6 remained lower, whereas the abundance of IL-1ra, IL-13, and MIP-1alpha increased. Changes in the signaling pathways involved in the Toll-like receptor signaling pathway, TNF signaling pathway, cytokine–cytokine receptor interaction, IL-17 signaling pathway, cytosolic DNA-sensing pathway, viral protein interaction with cytokine and cytokine receptor, inflammatory bowel disease, and JAK/STAT signaling pathway were found in patients with PACG compared to the control group.

The molecular changes in humans cannot be examined in a standardized manner, whereas using experimental glaucoma models allows the precise molecular and cellular changes to be investigated in a standardized manner. The second objective was to profile the cytokines in the retina of an *ex vivo* glaucoma animal model and then compare the cytokine profiles of the PACG patients and the animal model.

Although *ex vivo* models do not represent the intricacy of glaucomatous disease [29,30], subjecting retinal explants from rodents to elevated hydrostatic pressure is able to simulate some of the milieu of the retinal ganglion cells under glaucoma as well as the complexity of the internal cellular processes. This model has been used repeatedly in our



Pos	Pos	Neg	Neg	Activin A	Agrin	B7-2/CD86	b-NGF	CINC-1	CINC-2α	CNC-3	CNTF
Pos	Pos	Neg	Neg	Activin A	Agrin	B7-2/CD86	b-NGF	CINC-1	CINC-2α	CNC-3	CNTF
Fas Ligand	Fractalkine	GM-CSF	ICAM-1	IFN-γ	IL-1α	IL-1β	IL-1 R6	IL-2	IL-4	IL-6	IL-10
Fas Ligand	Fractalkine	GM-CSF	ICAM-1	IFN-γ	IL-1α	IL-1β	IL-1 R6	IL-2	IL-4	IL-6	IL-10
IL-13	Leptin	LIX	L-Selectin	MCP-1	MIP-3α	MMP-8	PDGF-AA	Prolactin R	RAGE	Thymus Chemokine-1	TIMP-1
IL-13	Leptin	LIX	L-Selectin	MCP-1	MIP-3α	MMP-8	PDGF-AA	Prolactin R	RAGE	Thymus Chemokine-1	TIMP-1
TNF-α	VEGF	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Pos
TNF-α	VEGF	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Pos

Fig. 4. Cytokine profiling of retinal samples from the glaucoma animal model and controls. (A) Representative cytokine antibody array membranes of the controls and the glaucoma model. (B) Heat map depicts the hierarchical clustering of the differentially expressed cytokines in the glaucoma model compared to the control group. (C) Eight cytokines were significantly upregulated in the glaucoma model (fold change cutoff of >1.5, n = 3). (D) Seven cytokines were significantly downregulated in the glaucoma model (fold change cutoff of <2/3, n = 3). $^{*}p < 0.05$.

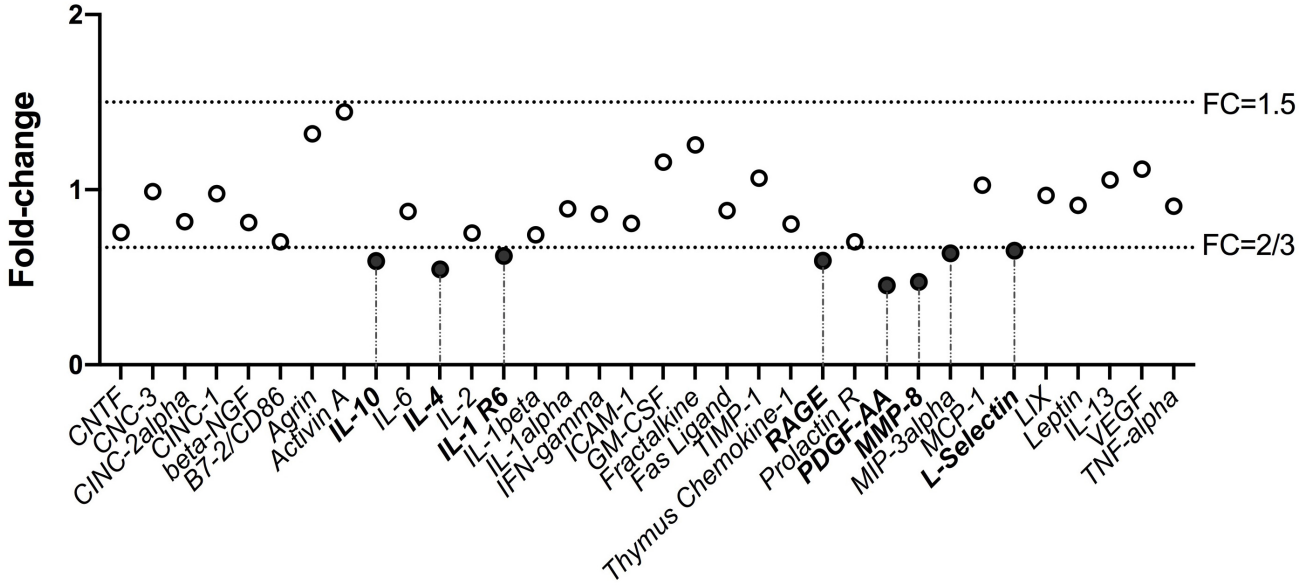


Fig. 5. Cytokine array of culture medium from glaucoma animal model and controls. Eight cytokines were downregulated in the culture medium of the glaucoma model compared to the control (fold change cutoff of <2/3, n = 3).

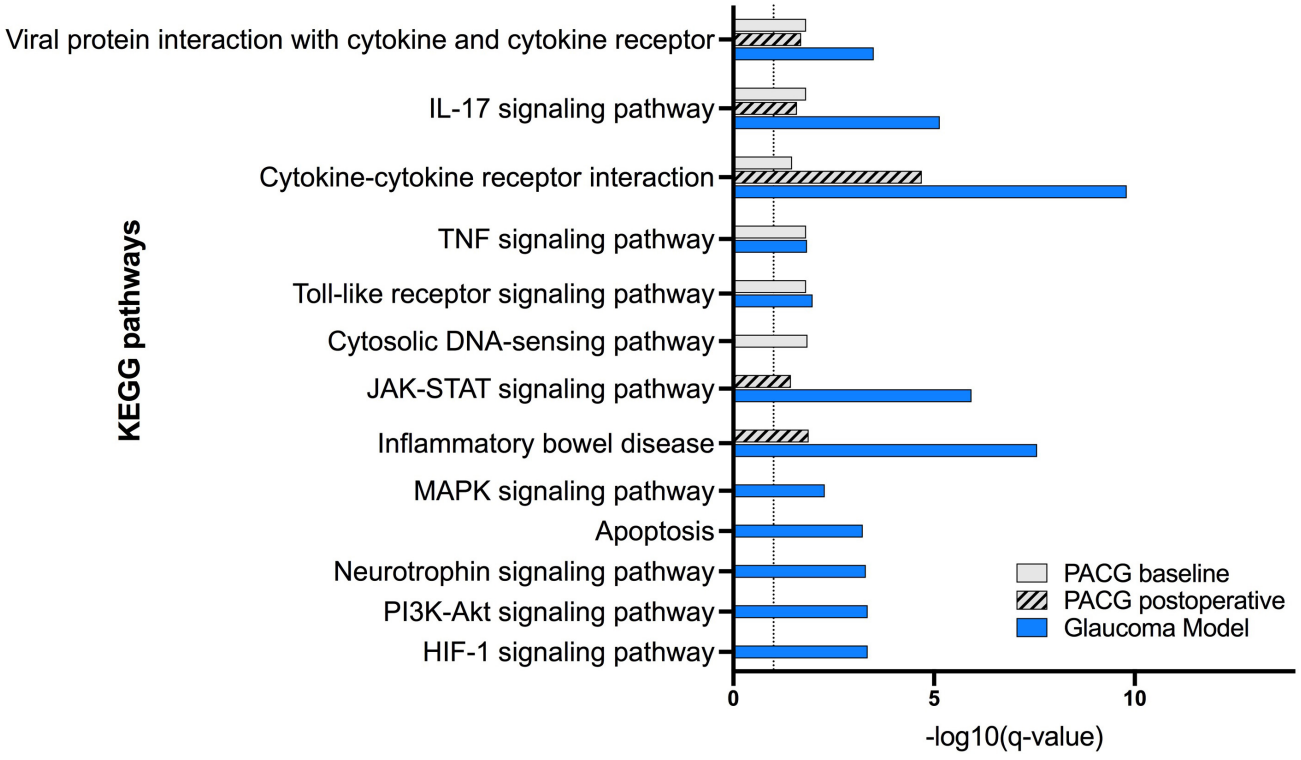


Fig. 6. Functional Enrichment Analysis of Significantly Altered Cytokines in PACG Patients and Glaucoma Animal Model. The list of the differentially expressed cytokines subjected to STRING analysis to reveal functional interaction.

previous studies to study the pathogenesis of glaucoma and to test potential neuroprotective treatments [31–35]. To assess how well the results obtained from this model can be transferred to glaucoma in humans, a better understanding of the similarities and differences between the *ex vivo* model and glaucoma patients is required. Culturing under fluctuating hydrostatic pressure led to significant loss of RGCs and changes in the cytokine profile. The abundance of CNTF, CINC-2α, IL-4, IL-6, IL-10, Fas ligand, b-NGF, and TIMP-1 was increased, while IFN-γ, TNF-α, MMP-8, L-selectin, RAGE, PDGF-AA, and prolactin R were decreased in the retinal samples from the glaucoma model.

In our study, we found that patients with PACG have some degree of inflammatory factor change in their eyes

before surgery. The level of IL-6 was decreased in PACG patients both at baseline and postoperatively. A meta-analysis concluded an overall reduction in AH IL-6 levels in patients with POAG and pseudoexfoliative glaucoma (PXG) compared to controls [36,37]. However, IL-6 was increased in the *ex vivo* model, as well as in early optic nerve head injury and inflammatory stimulation [38,39]. Perhaps the difference in the course of the diseases and properties of IL-6 could explain the discrepancy since it is a pleiotropic cytokine that possesses proinflammatory and antiapoptotic properties. IL-6 plays a central role in host defense mechanisms by regulating acute phase reactions. It has been reported to protect retinal ganglion cells from pressure-induced apoptosis by counteracting proapoptotic stimuli from retinal glial cells [38,40,41]. In addition to IL-6 itself, proteins related to IL-6, such as the CNTF, influence the survival of retinal cells [42], which is also significantly increased in the glaucoma model in this study. CNTF prolongs the survival of retinal ganglion cells against different injuries [43–46]. Both IL-6 and CNTF promote axonal growth in retinal ganglion cells in optic nerve crush or transection models [39,47,48].

Interferon-induced protein 10 (IP-10) is another cytokine that was significantly decreased in the AH of PACG patients at baseline. IP-10 belongs to the CXC chemokine family, which can induce chemotaxis, cell growth, apoptosis, angiogenesis, and inflammation. In other studies, the IP-10 level was found to be increased in the AH of patients with chronic PACG, as well as in patients with acute angle closure glaucoma (AACG) [49,50]. However, the exact role of IP-10 in glaucoma pathology is largely unclear.

Platelet-derived growth factor-BB (PDGF-BB) was significantly upregulated in the PACG group, whereas in the *ex vivo* model, PDGF-AA was significantly downregulated in both the retinal samples and the culture medium. The expression level of the PDGF family increased under hypoxic conditions and is believed to be associated with angiogenesis under hypoxia [51]. PDGF-BB is a potent inducer of proinflammatory cytokines [52]. In AACG patients who underwent trabeculectomy, those with consistently elevated IOP had significantly increased PDGF-CC and -DD levels in their AH compared to those with reduced IOP after trabeculectomy [53]. However, despite controlling IOP, glaucoma patients still had higher PDGF-BB in their tear samples as well as in their AH [54,55]. The PDGF-AA level was downregulated in the serum of PXG patients [37], yet upregulated in the AH of patients with neovascular glaucoma, phakic POAG, and phakic exfoliation glaucoma [56,57]. PDGF-AA has shown neuroprotective effects on RGCs both *in vitro* and *in vivo* in a hypertension model, while it also inhibits hydrogen peroxide-induced retinal cell death [58].

The AH cytokine profile in the second eye after the first eye cataract surgery reflects the inflammatory condition in PACG patients. IL-6 remained significantly decreased, while the levels of PDGF-BB and IP-10 were not significantly different compared to the control. The levels of IL-1ra, IL-13, and MIP-1α were significantly increased in the PACG group compared to the control. IL-1ra was also found to be increased in the AH of POAG patients [59]. The interleukin-1 (IL-1) family is a central mediator of innate immunity and inflammation [60], and has been linked to neurodegenerative and neuroinflammatory diseases, such as Alzheimer's disease, Parkinson's disease, acute neurodegeneration in the central nervous system, and retinal degenerative diseases [61–64]. IL-1ra is an anti-inflammatory competitive receptor antagonist [65–67]. An age-related increase in IL-1ra was also observed in donor eyes [68]. The levels of IL-1α and IL-1β were found to be increased in the trabecular meshwork of POAG patients and seem to play a role in glaucoma pathology. IL-1ra also protects trabecular meshwork (TM) cells against oxidative stress [69,70]. IL-13 is another anti-inflammatory cytokine that was also significantly increased in the AH in this study, as well as in another study on PACG patients [71]. IL-13 is associated with AMD and proliferative diabetic retinopathy [72,73]. However, its role in glaucoma and RGCs has been less studied. MIP-1α was also significantly higher in the AH in this study and in the AH from POAG eyes [55]. Macrophage inflammatory protein-1alpha (MIP-1α) actors produced by macrophages and monocytes after they are stimulated with proinflammatory cytokines, such as IL-1β, are crucial for immune responses towards infection and inflammation [74]. These results indicate that PACG patients show stronger intraocular inflammatory reactions toward topical stimuli compared with the controls.

It is clear that cytokine levels vary among studies in patients with the same diagnosis, in not only the different stages and severity of disease but also the variability in ages, treatments of the recruited patients, and the method used to analyze the cytokines have a distinct impact on the results. Commonalities among studies are that glaucoma patients have shown distinguished inflammatory response profiles in their AH compared to the control. Indeed, similar changes are observed in the signaling pathways in the retinal samples from the glaucoma model to patients with PACG, such as in the Toll-like receptor signaling pathway, TNF signaling pathway, cytokine–cytokine receptor interaction, IL-17 signaling pathway, and JAK/STAT signaling pathway; furthermore, there are also changes in pathways seen exclusively in the glaucoma model, such as in the MAPK signaling, apoptosis, neurotrophin signaling, PI3k-Ark signaling, and HIF-1 signaling pathways. However, the mechanisms involved need further investigation.

Human retinal samples are difficult to obtain. The AH is the most frequently sampled ocular material in examinations and clinical studies of ocular disease, including diseases in the posterior segment of the eye. It is widely assumed by ophthalmology researchers that the AH cytokine levels are influenced by the retina. The AH cytokine levels

have been reported to be correlated with retinal conditions, such as age-related macular degeneration, diabetic macular edema, chronic central serous chorioretinopathy, and glaucoma [19,75–81].

In this study, the cytokine changes in the culture medium partially reflect the cytokine changes in the retinal samples. A relative decrease in the abundance of IL-10, IL-4, IL-1r6, MMP-8, L-selectin, RAGE, and PDGF-AA was observed in the culture medium. In our previous study, changes in the retinal proteome were also detected in the vitreous body in an *in vivo* glaucoma model [33]. Due to the size of the rodent eyeball, it is difficult to collect the AH from small animals, the exact correlation between cytokines in the retina and the AH needs to be explored in bigger animals.

To the best of our knowledge, this is the first study to compare the cytokine profiles of glaucoma patients with an *ex vivo* model. The alterations in cytokine profiles seem to be quite different in the retinal samples from the glaucoma model and the AH samples from PACG patients. This is probably because the *ex vivo* model represents an acute phase of retinal degeneration, while the AH samples were not collected immediately after the operation. Nonetheless, similar cytokine changes in the *ex vivo* glaucoma model can be seen in the AH from glaucoma patients in other studies. For instance, an increase in IFN-γ and MIP-1α was found in the AH, while IL-4 was increased in the tears of POAG patients [55]. The levels of PXG, fractalkine, IL-10, and IL1Ra were significantly upregulated in serum, while the level of PDGF-AA was downregulated [37].

5. Conclusions

The different cytokines between patients with PACG and the controls confirm that eyes with PACG retained a mild inflammation and in response to external stimulation, such as the cataract surgery, a stronger inflammatory reaction was also observed in eyes with PACG. The cytokine profile for the culture medium partially represents the cytokine changes in the retina. The glaucoma model simulates similar cytokine and pathway changes not only in PACG patients but also in AACG, POAG, and PXG. It is a practical tool to study some of the specific changes involved in glaucoma pathology. However, it is also important to address the differences between this model and glaucoma patients, thus, caution should be taken when translating the results from this model to the glaucoma pathology in human patients.

Availability of Data and Materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Author Contributions

JT and YF performed the measurements, HC, VP and HL were involved in planning and supervised the work, JT, MS, YF and HL processed the experimental data, performed the analysis, drafted the manuscript and designed the figures. JT, MS, VP and HL wrote the paper with input from all authors. All authors discussed the results and commented on the manuscript. All authors read and approved the final manuscript. All authors contributed to editorial changes in the manuscript. All authors have participated sufficiently in the work and agreed to be accountable for all aspects of the work.

Ethics Approval and Consent to Participate

The study was performed according to local ethical approval protocol no. EC. D (BG) .016.03.1-2018-007. Informed consent was obtained from all subjects enrolled in the study. The study was in accordance with the guidelines of the ethical commission of Shanghai East Hospital of Tongji University.

Acknowledgment

Not applicable.

Funding

This research was funded by National Natural Science Foundation of China (81870634) and Deutsche Forschungsgemeinschaft (DFG), grant number PR1569-1-1.

Conflict of Interest

The authors declare no conflict of interest.

References

[1] Weinreb RN, Aung T, Medeiros FA. The pathophysiology and treatment of glaucoma: a review. JAMA. 2014; 311: 1901–1911.

[2] Weinreb RN, Kaufman PL. Glaucoma research community and FDA look to the future, II: NEI/FDA Glaucoma Clinical Trial Design and Endpoints Symposium: measures of structural change and visual function. Investigative Ophthalmology & Visual Science. 2011; 52: 7842–7851.

[3] Heneka MT, Carson MJ, El Khoury J, Landreth GE, Brosseron F, Feinstein DL, *et al*. Neuroinflammation in Alzheimer's disease. The Lancet. Neurology. 2015; 14: 388–405.

[4] Xu H, Chen M, Forrester JV. Para-inflammation in the aging retina. Progress in Retinal and Eye Research. 2009; 28: 348–368.

[5] Baltmr A, Duggan J, Nizari S, Salt TE, Cordeiro MF. Neuroprotection in glaucoma - Is there a future role? Experimental Eye Research. 2010; 91: 554–566.

[6] Soto I, Howell GR. The complex role of neuroinflammation in glaucoma. Cold Spring Harbor Perspectives in Medicine. 2014; 4: a017269.

[7] Vernazza S, Tirendi S, Bassi AM, Traverso CE, Saccà SC. Neuroinflammation in Primary Open-Angle Glaucoma. Journal of Clinical Medicine. 2020; 9: 3172.

[8] Freedman J, Iserovich P. Pro-inflammatory cytokines in glaucomatous aqueous and encysted Molteno implant blebs and their relationship to pressure. Investigative Ophthalmology & Visual Science. 2013; 54: 4851–4855.
[9] Sawada H, Fukuchi T, Tanaka T, Abe H. Tumor necrosis factor-alpha concentrations in the aqueous humor of patients with glaucoma. Investigative Ophthalmology & Visual Science. 2010; 51: 903–906.
[10] Zenkel M, Lewczuk P, Jünemann A, Kruse FE, Naumann GOH, Schlötzer-Schrehardt U. Proinflammatory cytokines are involved in the initiation of the abnormal matrix process in pseudoexfoliation syndrome/glaucoma. The American Journal of Pathology. 2010; 176: 2868–2879.
[11] Chen KH, Wu CC, Roy S, Lee SM, Liu JH. Increased interleukin-6 in aqueous humor of neovascular glaucoma. Investigative Ophthalmology & Visual Science. 1999; 40: 2627–2632.
[12] Hu DN, Ritch R, Liebmann J, Liu Y, Cheng B, Hu MS. Vascular endothelial growth factor is increased in aqueous humor of glaucomatous eyes. Journal of Glaucoma. 2002; 11: 406–410.
[13] Kuchtey J, Rezaei KA, Jaru-Ampornpan P, Sternberg P, Jr, Kuchtey RW. Multiplex cytokine analysis reveals elevated concentration of interleukin-8 in glaucomatous aqueous humor. Investigative Ophthalmology & Visual Science. 2010; 51: 6441–6447.
[14] Chua J, Vania M, Cheung CMG, Ang M, Chee SP, Yang H, *et al*. Expression profile of inflammatory cytokines in aqueous from glaucomatous eyes. Molecular Vision. 2012; 18: 431–438.
[15] Prendes MA, Harris A, Wirostko BM, Gerber AL, Siesky B. The role of transforming growth factor β in glaucoma and the therapeutic implications. The British Journal of Ophthalmology. 2013; 97: 680–686.
[16] Park YG, Park WK, Kim RY, Kim M, Park YH. Serial changes in the aqueous IL-10 level after intravitreal methotrexate injection as an indicator of primary vitreoretinal lymphoma recurrence. Scientific Reports. 2020; 10: 15992.
[17] Takeda A, Hasegawa E, Nakao S, Ishikawa K, Murakami Y, Hisatomi T, *et al*. Vitreous levels of interleukin-35 as a prognostic factor in B-cell vitreoretinal lymphoma. Scientific Reports. 2020; 10: 15715.
[18] Sato T, Enoki T, Karasawa Y, Someya H, Taguchi M, Harimoto K, *et al*. Inflammatory Factors of Macular Atrophy in Eyes With Neovascular Age-Related Macular Degeneration Treated With Aflibercept. Frontiers in Immunology. 2021; 12: 738521.
[19] Mao J, Zhang S, Zheng Z, Deng X, Liu C, Chen Y, *et al*. Prediction of anti-VEGF efficacy in diabetic macular oedema using intraocular cytokines and macular optical coherence tomography. Acta Ophthalmologica. 2022; 100: e891–e898.
[20] Dietz JA, Maes ME, Huang S, Yandell BS, Schlamp CL, Montgomery AD, *et al*. Spink2 modulates apoptotic susceptibility and is a candidate gene in the Rgcs1 QTL that affects retinal ganglion cell death after optic nerve damage. PLoS ONE. 2014; 9: e93564.
[21] Li Y, Semaan SJ, Schlamp CL, Nickells RW. Dominant inheritance of retinal ganglion cell resistance to optic nerve crush in mice. BMC Neuroscience. 2007; 8: 19.
[22] Templeton JP, Freeman NE, Nickerson JM, Jablonski MM, Rex TS, Williams RW, *et al*. Innate immune network in the retina activated by optic nerve crush. Investigative Ophthalmology & Visual Science. 2013; 54: 2599–2606.
[23] Samsel PA, Kisiswa L, Erichsen JT, Cross SD, Morgan JE. A novel method for the induction of experimental glaucoma using magnetic microspheres. Investigative Ophthalmology & Visual Science. 2011; 52: 1671–1675.
[24] Sappington RM, Carlson BJ, Crish SD, Calkins DJ. The microbead occlusion model: a paradigm for induced ocular hypertension in rats and mice. Investigative Ophthalmology & Visual Science. 2010; 51: 207–216.
[25] Tannenbaum J, Bennett BT. Russell and Burch's 3Rs then and now: the need for clarity in definition and purpose. Journal of the American Association for Laboratory Animal Science. 2015; 54: 120–132.
[26] Schnichels S, Kiebler T, Hurst J, Maliha AM, Löscher M, Dick HB, *et al*. Retinal Organ Cultures as Alternative Research Models. Alternatives to Laboratory Animals. 2019; 47: 19–29.
[27] Foster PJ, Buhrmann R, Quigley HA, Johnson GJ. The definition and classification of glaucoma in prevalence surveys. The British Journal of Ophthalmology. 2002; 86: 238–242.
[28] Szklarczyk D, Gable AL, Lyon D, Junge A, Wyder S, Huerta-Cepas J, *et al*. STRING v11: protein-protein association networks with increased coverage, supporting functional discovery in genome-wide experimental datasets. Nucleic Acids Research. 2019; 47: D607–D613.
[29] Danesh-Meyer HV, Levin LA. Neuroprotection: extrapolating from neurologic diseases to the eye. American Journal of Ophthalmology. 2009; 148: 186–191.e2.
[30] Kimura A, Noro T, Harada T. Role of animal models in glaucoma research. Neural Regeneration Research. 2020; 15: 1257–1258.
[31] Prokosch V, Brockhaus K, Anders F, Liu H, Mercieca K, Gericke A, *et al*. Elevated intraocular pressure induces neuron-specific β-III-tubulin expression in non-neuronal vascular cells. Acta Ophthalmologica. 2020; 98: e617–e630.
[32] Mann C, Thanos S, Brockhaus K, Grus FH, Pfeiffer N, Prokosch V. Endothelial Cell Reaction to Elevated Hydrostatic Pressure and Oxidative Stress in Vitro. Klinische Monatsblatter fur Augenheilkunde. 2019; 236: 1122–1128.
[33] Anders F, Mann C, Liu A, Teister J, Funke S, Thanos S, *et al*. Correlation of Crystallin Expression and RGC Susceptibility in Experimental Glaucoma Rats of Different Ages. Current Eye Research. 2018; 43: 1267–1273.
[34] Anders F, Liu A, Mann C, Teister J, Lauzi J, Thanos S, *et al*. The Small Heat Shock Protein α-Crystallin B Shows Neuroprotective Properties in a Glaucoma Animal Model. International Journal of Molecular Sciences. 2017; 18: 2418.
[35] Huang S, Huang P, Liu X, Lin Z, Wang J, Xu S, *et al*. Relevant variations and neuroprotecive effect of hydrogen sulfide in a rat glaucoma model. Neuroscience. 2017; 341: 27–41.
[36] Ulhaq ZS, Soraya GV. Aqueous humor interleukin-6 levels in primary open-angle glaucoma (POAG): A systematic review and meta-analysis. Archivos de la Sociedad Espanola de Oftalmologia. 2020; 95: 315–321.
[37] Sahay P, Reddy S, Prusty BK, Modak R, Rao A. TGFβ1, MMPs and cytokines profiles in ocular surface: Possible tear biomarkers for pseudoexfoliation. PLoS ONE. 2021; 16: e0249759.
[38] Sappington RM, Chan M, Calkins DJ. Interleukin-6 protects retinal ganglion cells from pressure-induced death. Investigative Ophthalmology & Visual Science. 2006; 47: 2932–2942.
[39] Leibinger M, Müller A, Gobrecht P, Diekmann H, Andreadaki A, Fischer D. Interleukin-6 contributes to CNS axon regeneration upon inflammatory stimulation. Cell Death & Disease. 2013; 4: e609.
[40] Thier M, März P, Otten U, Weis J, Rose-John S. Interleukin-6 (IL-6) and its soluble receptor support survival of sensory neurons. Journal of Neuroscience Research. 1999; 55: 411–422.
[41] Conroy SM, Nguyen V, Quina LA, Blakely-Gonzales P, Ur C, Netzeband JG, *et al*. Interleukin-6 produces neuronal loss in developing cerebellar granule neuron cultures. Journal of Neuroimmunology. 2004; 155: 43–54.
[42] Sarup V, Patil K, Sharma SC. Ciliary neurotrophic factor and its receptors are differentially expressed in the optic nerve transected adult rat retina. Brain Research. 2004; 1013: 152–158.
[43] Meyer-Franke A, Kaplan MR, Pfrieger FW, Barres BA. Charac-

terization of the signaling interactions that promote the survival and growth of developing retinal ganglion cells in culture. Neuron. 1995; 15: 805–819.
[44] Takahata K, Katsuki H, Kume T, Nakata D, Ito K, Muraoka S, *et al*. Retinal neuronal death induced by intraocular administration of a nitric oxide donor and its rescue by neurotrophic factors in rats. Investigative Ophthalmology & Visual Science. 2003; 44: 1760–1766.
[45] van Adel BA, Kostic C, Déglon N, Ball AK, Arsenijevic Y. Delivery of ciliary neurotrophic factor via lentiviral-mediated transfer protects axotomized retinal ganglion cells for an extended period of time. Human Gene Therapy. 2003; 14: 103–115.
[46] MacLaren RE, Buch PK, Smith AJ, Balaggan KS, MacNeil A, Taylor JS, *et al*. CNTF gene transfer protects ganglion cells in rat retinae undergoing focal injury and branch vessel occlusion. Experimental Eye Research. 2006; 83: 1118–1127.
[47] Cui Q, Lu Q, So KF, Yip HK. CNTF, not other trophic factors, promotes axonal regeneration of axotomized retinal ganglion cells in adult hamsters. Investigative Ophthalmology & Visual Science. 1999; 40: 760–766.
[48] Jo SA, Wang E, Benowitz LI. Ciliary neurotrophic factor is an axogenesis factor for retinal ganglion cells. Neuroscience. 1999; 89: 579–591.
[49] Duvesh R, Puthuran G, Srinivasan K, Rengaraj V, Krishnadas SR, Rajendrababu S, *et al*. Multiplex Cytokine Analysis of Aqueous Humor from the Patients with Chronic Primary Angle Closure Glaucoma. Current Eye Research. 2017; 42: 1608–1613.
[50] Tong Y, Zhou YL, Zheng Y, Biswal M, Zhao PQ, Wang ZY. Analyzing cytokines as biomarkers to evaluate severity of glaucoma. International Journal of Ophthalmology. 2017; 10: 925–930.
[51] Arjamaa O, Nikinmaa M. Oxygen-dependent diseases in the retina: role of hypoxia-inducible factors. Experimental Eye Research. 2006; 83: 473–483.
[52] van Steensel L, Paridaens D, Dingjan GM, van Daele PLA, van Hagen PM, Kuijpers RWAM, *et al*. Platelet-derived growth factor-BB: a stimulus for cytokine production by orbital fibroblasts in Graves' ophthalmopathy. Investigative Ophthalmology & Visual Science. 2010; 51: 1002–1007.
[53] Wang J, Fu MS, Zhou MW, Ke BL, Zhang ZH, Xu X. Potential effects of angiogenesis-related factors on the severity of APAC and surgical outcomes of trabeculectomy. BMC Ophthalmology. 2021; 21: 297.
[54] Martinez-de-la-Casa JM, Perez-Bartolome F, Urcelay E, Santiago JL, Moreno-Montanes J, Arriola-Villalobos P, *et al*. Tear cytokine profile of glaucoma patients treated with preservative-free or preserved latanoprost. The Ocular Surface. 2017; 15: 723–729.
[55] Burgos-Blasco B, Vidal-Villegas B, Saenz-Frances F, Morales-Fernandez L, Perucho-Gonzalez L, Garcia-Feijoo J, *et al*. Tear and aqueous humour cytokine profile in primary open-angle glaucoma. Acta Ophthalmologica. 2020; 98: e768–e772.
[56] Ohira S, Inoue T, Shobayashi K, Iwao K, Fukushima M, Tanihara H. Simultaneous increase in multiple proinflammatory cytokines in the aqueous humor in neovascular glaucoma with and without intravitreal bevacizumab injection. Investigative Ophthalmology & Visual Science. 2015; 56: 3541–3548.
[57] Inoue T, Kawaji T, Inatani M, Kameda T, Yoshimura N, Tanihara H. Simultaneous increases in multiple proinflammatory cytokines in the aqueous humor in pseudophakic glaucomatous eyes. Journal of Cataract and Refractive Surgery. 2012; 38: 1389–1397.
[58] Kanamoto T, Rimayanti U, H O, Kiuchi Y. Platelet-derived growth factor receptor alpha is associated with oxidative stress-induced retinal cell death. Current Eye Research. 2011; 36: 336–340.
[59] Pantalon A, Obadă O, Constantinescu D, Feraru C, Chiseliță D. Inflammatory model in patients with primary open angle glaucoma and diabetes. International Journal of Ophthalmology. 2019; 12: 795–801.
[60] Dinarello CA. Overview of the IL-1 family in innate inflammation and acquired immunity. Immunological Reviews. 2018; 281: 8–27.
[61] Shaftel SS, Griffin WST, O'Banion MK. The role of interleukin-1 in neuroinflammation and Alzheimer disease: an evolving perspective. Journal of Neuroinflammation. 2008; 5: 7.
[62] Patel HC, Boutin H, Allan SM. Interleukin-1 in the brain: mechanisms of action in acute neurodegeneration. Annals of the New York Academy of Sciences. 2003; 992: 39–47.
[63] Allan SM, Tyrrell PJ, Rothwell NJ. Interleukin-1 and neuronal injury. Nature Reviews. Immunology. 2005; 5: 629–640.
[64] McGeer PL, Yasojima K, McGeer EG. Association of interleukin-1 beta polymorphisms with idiopathic Parkinson's disease. Neuroscience Letters. 2002; 326: 67–69.
[65] Chatziralli I, Sergentanis TN, Crosby-Nwaobi R, Winkley K, Eleftheriadis H, Ismail K, *et al*. Model for Risk-Based Screening of Diabetic Retinopathy in People With Newly-Diagnosed Type 2 Diabetes Mellitus. Investigative Ophthalmology & Visual Science. 2017; 58: BIO99–BIO105.
[66] Scuderi S, D'amico AG, Federico C, Saccone S, Magro G, Bucolo C, *et al*. Different Retinal Expression Patterns of IL-1α, IL-1β, and Their Receptors in a Rat Model of Type 1 STZ-Induced Diabetes. Journal of Molecular Neuroscience: MN. 2015; 56: 431–439.
[67] Liu J, Shi B, He S, Yao X, Willcox MDP, Zhao Z. Changes to tear cytokines of type 2 diabetic patients with or without retinopathy. Molecular Vision. 2010; 16: 2931–2938.
[68] Cao S, Walker GB, Wang X, Cui JZ, Matsubara JA. Altered cytokine profiles of human retinal pigment epithelium: oxidant injury and replicative senescence. Molecular Vision. 2013; 19: 718–728.
[69] Wang N, Chintala SK, Fini ME, Schuman JS. Activation of a tissue-specific stress response in the aqueous outflow pathway of the eye defines the glaucoma disease phenotype. Nature Medicine. 2001; 7: 304–309.
[70] Mookherjee S, Banerjee D, Chakraborty S, Banerjee A, Mukhopadhyay I, Sen A, *et al*. Association of IL1A and IL1B loci with primary open angle glaucoma. BMC Medical Genetics. 2010; 11: 99.
[71] de Vries JE. The role of IL-13 and its receptor in allergy and inflammatory responses. The Journal of Allergy and Clinical Immunology. 1998; 102: 165–169.
[72] Fu B, Liu ZL, Zhang H, Gu F. Interleukin-13 and age-related macular degeneration. International Journal of Ophthalmology. 2017; 10: 535–540.
[73] Yoshida S, Kobayashi Y, Nakama T, Zhou Y, Ishikawa K, Arita R, *et al*. Increased expression of M-CSF and IL-13 in vitreous of patients with proliferative diabetic retinopathy: implications for M2 macrophage-involving fibrovascular membrane formation. The British Journal of Ophthalmology. 2015; 99: 629–634.
[74] Ren M, Guo Q, Guo L, Lenz M, Qian F, Koenen RR, *et al*. Polymerization of MIP-1 chemokine (CCL3 and CCL4) and clearance of MIP-1 by insulin-degrading enzyme. The EMBO Journal. 2010; 29: 3952–3966.
[75] Errera MH, Pratas A, Fisson S, Manicom T, Boubaya M, Sedira N, *et al*. Cytokines, chemokines and growth factors profile in human aqueous humor in idiopathic uveitis. PloS One. 2022; 17: e0254972.
[76] Mao J, Zhang C, Zhang S, Liu C, Chen N, Tao J, *et al*. Predictors of anti-VEGF efficacy in chronic central serous chori-

oretinopathy based on intraocular cytokine levels and pigment epithelium detachment subtypes. Acta Ophthalmologica. 2022; 100: e1385–e1394.

[77] Xu Q, Gong C, Qiao L, Feng R, Liu H, Liu Y, *et al*. Aqueous Level of ANGPTL4 Correlates with the OCTA Metrics of Diabetic Macular Edema in NPDR. Journal of Diabetes Research. 2022; 2022: 8435603.

[78] Pinazo-Durán MD, Zanón-Moreno V, García-Medina JJ, Gallego-Pinazo R. Evaluation of presumptive biomarkers of oxidative stress, immune response and apoptosis in primary open-angle glaucoma. Current Opinion in Pharmacology. 2013; 13: 98–107.

[79] Azbukina NV, Chistyakov DV, Goriainov SV, Kotelin VI, Fedoseeva EV, Petrov SY, *et al*. Targeted Lipidomic Analysis of Aqueous Humor Reveals Signaling Lipid-Mediated Pathways in Primary Open-Angle Glaucoma. Biology. 2021; 10: 658.

[80] Liu X, Liu X, Wang Y, Sun H, Guo Z, Tang X, *et al*. Proteome Characterization of Glaucoma Aqueous Humor. Molecular & Cellular Proteomics. 2021; 20: 100117.

[81] Igarashi N, Honjo M, Yamagishi R, Kurano M, Yatomi Y, Igarashi K, *et al*. Crosstalk between transforming growth factor β-2 and Autotaxin in trabecular meshwork and different subtypes of glaucoma. Journal of Biomedical Science. 2021; 28: 47.