Journal of Inflammation Research

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ORIGINAL RESEARCH

Alterations in Peripheral B Cell Subsets Correlate with the Disease Severity of Human Glaucoma

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Background: Glaucoma is a group of retinal neurodegenerative diseases causing irreversible visual impairment. The pathogenesis of this disease is complicated. Studies have shown that the immune system is involved in the neurodegenerative process of glaucoma. There are continuous evidences that autoantibodies play a crucial role in the pathogenesis of glaucoma. However, focuses on B cells, the antibody-producing cells in glaucoma are surprisingly limited.

Methods: Fresh peripheral blood samples were collected from 44 glaucoma patients (38 with primary angle-closure glaucoma (PACG) and 6 with (primary open-angle glaucoma POAG)) and 36 age-matched healthy donors (HD). Density gradient centrifugation was performed to obtain peripheral blood mononuclear cells (PBMC). Flow cytometry was performed to determine B cell phenotypes. The severity of glaucoma was determined based on the mean deviation (MD) of visual field.

Results: In this study, we demonstrated that total B cells was significantly increased in glaucoma patients compared to HD. Next, we checked changes of different B cell subsets in glaucoma. Glaucoma patients were found to have a significant increase in the frequencies of antibody-secreting cells (ASC)/plasmablasts, naïve, and $CD19^+$ $CD27^-$ IgD^- double negative (DN) subpopulations, but a decrease in the $CD27^+$ IgD^+ unswitched memory compartment. Notably, we found that the increment of $CD27^-$ IgD^- DN B cells was significantly magnified according to the clinical severity.

Conclusion: We demonstrate, for the first time, that peripheral B cell subsets are altered and unveil the correlation of a newly identified pro-inflammatory $CD27^-$ IgD^- DN subset with clinical features of glaucoma, suggesting that these B cell subsets could serve as potential biomarkers to monitor the disease progression of glaucoma patients.

Keywords: glaucoma, B cells, $CD27^-$ IgD^- double negative B cells, retinal neurodegeneration

Introduction

Glaucoma is a group of neurodegenerative diseases with a complex pathogenesis, which mainly affects the retinal ganglion cells (RGC) and optic nerve (ON), leading to irreversible visual impairment. There are about 70 million glaucoma patients in the world, about half of them in Asia, while nearly 10 million glaucoma patients in China.[1,2]

The pathogenesis of glaucoma is complicated and it is considered that high intraocular pressure (IOP) is the most important risk factor in the pathogenesis of glaucoma. IOP reduction surgery is still the most commonly used treatment for retinal damage of glaucoma,[3–5] but patients with elevated IOP do not necessarily suffer from RGC/ON damage, while patients with normal or effectively controlled

Received: 16 July 2021
Accepted: 16 September 2021
Published: 21 September 2021

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IOP might still have progressive neurodegenerative damage. Therefore, more and more studies begin to pay attention to other factors that might be related to retinal neurodegeneration besides IOP, in order to find novel therapeutic intervention.[6,7] It has been implicated that there are other factors related to the pathogenesis of glaucoma, such as vascular factors,[8] high myopia,[9,10] oxidative stress,[11] and immune responses.[6,12]

Increasing clinical and experimental studies have shown that an immunologic component is involved in the neurodegenerative process of glaucoma.[7] Studies have shown that the proliferation and activation of microglial cells (MGC) in the retina of glaucomatous mice are up-regulated, and the pharmacological intervention in the early stage of the disease is able to deactivate MGC, which is effective in reducing nerve injury.[13] In addition to innate immunity, the role of antibodies, which belong to the adaptive arm of the immune system, in the pathogenesis of glaucoma has also attracted much attention.[14,15] Two decades ago, Wax's team discovered that antibody against HSP60 was up-regulated in the serum of glaucoma patients and implicated that this antibody might be involved in the glaucomatous optic neuropathy process in an IOP-independent manner.[16–18] Since then, different studies have revealed a variety of autoantibodies against retinal and ON antigens have been altered in sera and aqueous humour of glaucoma patients.[19] For example, antibodies against HSP70,[20] α-fodrin,[21] myelin basic protein (MBP),[22] or HSP27[23] are significantly up-regulated, while those against glial fibrillary acidic protein (GFAP)[22] or vimentin[20] are down-regulated. Amounts of studies have emphasized the importance of autoantibodies in the pathogenesis of glaucoma. In 1998, Wax and the team demonstrated, for the first time, that antibodies were accumulated in the retina of a patients with NTG[24] and this was further verified by a following study showing RGC loss was accompanied by evident autoantibody deposits in 4 glaucomatous donor retinas.[25] Studies on an experimental glaucoma animal model induced by specific antigen immunization revealed similar alterations of antibody profiles compared to those in human glaucoma.[26–28] Of note, RGC and ON degeneration was observed to be often accompanied with antibody deposits and occur in the areas where the antibodies were accumulated.[29–31] Besides pathogenic roles, some antibodies have been implicated to have neuroprotective potential. Several decreased antibodies in human glaucoma have been identified to be protective against stress factors, which have been implicated in the pathogenesis of glaucoma.[11] For instance, in vitro preincubation of RGC-5 (a neuroretinal cell line) with antibody against the protein kinase inhibitor 14-3-3 or γ-synuclein evidently abrogates reactive oxygen species (ROS) generation induced by different stress factors and increases cell viability.[32,33] Although great progresses have been made in the understanding of autoantibodies in glaucoma, focuses on B cells, the antibody-producing cells, in glaucoma are surprisingly limited. Previous studies have shown that B cell infiltration in the retina of glaucoma patients, but no reports have explored the B cell subset alteration in the peripheral blood of glaucoma patients.

In this study, we enrolled 44 patients with glaucoma and 36 age-matched healthy donors (HD), collected their peripheral blood, and performed flow cytometry to determine the alterations of total B cells and their subsets in the peripheral blood of glaucoma patients. We found that total B cells, antibody-secreting cells (ASC)/plasmablasts, naïve, and $CD19^{+}$ $CD27^{-}$ IgD^{-} double negative (DN) subpopulations were significantly up-regulated, while the unswitched memory compartment was remarkably down-regulated. Notably, we found that the $CD27^{-}$ IgD^{-} DN subset was positively associated with the clinical severity of glaucoma. We here, for the first time, demonstrate that peripheral B cell subsets are altered and unveils the correlation of a newly identified pro-inflammatory $CD27^{-}$ IgD^{-} DN subset with clinical severity of glaucoma, suggesting that these B cell subsets could serve as potential biomarkers to monitor the disease progression of glaucoma patients.

Materials and Methods

Subjects

Our study was conducted in accordance with the Declaration of Helsinki and approved by the Institutional Review Board for Clinical Research of the Daping Hospital (No. 202192), and Sichuan Provincial People's Hospital (No. 201968). All subjects were well informed about the study and potential risk and signed an informed consent before participation. To investigate the subset distribution and functional changes of B cells in human glaucoma, we enrolled 44 patients with glaucoma (38 with primary angle-closure glaucoma (PACG) and 6 with (primary open-angle glaucoma POAG)) and 36 age-matched healthy donors (HD) from the Department of Ophthalmology, Daping Hospital (Chongqing, China).

All subjects (glaucoma patients and HD) underwent the standard workup for glaucoma, including medical history, physical and ophthalmologic examination, visual field tests and optical coherence tomography (OCT). Glaucoma was diagnosed according to the combination of age, family history, glaucomatous clinical manifestation, IOP elevation, cup to disk ratio, visual field loss and the thickness of RNFL. The exclusion criteria of glaucoma patients or HD who had no clinical evidence of glaucoma or a family history of glaucoma in this study: subjects with a history of other ocular diseases (such as uveitis, aged macular degeneration, cataracts, high myopia and retinitis pigmentosa), hypertension, diabetes, cardiovascular diseases, systemic autoimmune diseases or cancers, or subjects taking any systemic medications affecting the immune system were excluded. The severity of glaucoma was determined based on the mean deviation (MD) of visual field: mild indicates visual field MD of greater than −6 dB; moderate, −12 dB to −6 dB; and severe, no greater than −12 dB.[34] The demographics of subjects enrolled in this study were shown in Table 1.

Blood Sample Collection and Flow Cytometry

Peripheral blood (10 mL) was collected on the day when patients were hospitalized for surgery (before surgery) using sodium heparin-anticoagulated Vacutainer CPT tubes (BD Biosciences, San Diego, CA, USA). At the time of blood sampling, no subjects had an acute infection or were taking any medication known to influence immune function. Blood samples were immediately processed within 2 hours to obtain peripheral blood mononuclear cells (PBMC) as described previously.[35] Briefly, fresh blood samples were diluted 1:1 with Ca^{2+}/Mg^{2+} free phosphate buffered saline (PBS) and then overlaid onto Ficoll (Ficoll Paque; GE Healthcare, twice the volume of PBS-blood) in a 50 mL centrifuge tube. Density gradient centrifugation was then performed at room temperature for 20 minutes at 2000 rpm and the cloudy interface between ficoll and serum was collected to obtain PBMC. After being washed twice in cold Stain Buffer containing BSA (BD Biosciences, RRID: AB_2869007), freshly isolated PBMC were used for flow cytometric staining. PBMC (10^6 cells for each flow cytometric test) were divided into 4 tests: 3 replicates for B cell phenotype (the mean value of 3 replicates was shown in each figure) and 1 for corresponding isotype staining. To determine B cell phenotype, PBMC were stained for viability (LIVE/DEAD™ Fixable Near-IR Dead Cell Stain Kit, Invitrogen, Thermo Fisher Scientific, USA) and fluorochrome-conjugated antibodies (all antibodies and their matched isotype antibodies were purchased from BioLegend, San Diego, CA, USA) against CD19 (AB_314245), CD27 (AB_314297), CD38 (AB_2561901), IgD (AB_2561386), CD24 (AB_10962689) for 30 minutes. Matched isotype antibodies were used simultaneously to exclude non-specific antibody binding and spectral overlap. After washed by Stain Buffer, 1% paraformaldehyde was used to resuspend cells. Flow cytometric data were acquired using a FACS Canto II flow cytometer (BD Biosciences) and analyzed using FlowJo software (Tree Star, Ashland, OR, USA).

Statistical Analysis

The statistical analysis was performed by using a Prism software (GraphPad Software, San Diego, California, USA). Data are presented as mean ± SD. Unpaired Student's *t*-test (two-tailed) was performed to determine the statistic difference between 2 groups and $p<0.05$ was considered statistically significant. When the number of groups = 3, one-way analysis of variance (ANOVA) was applied. For the comparation between different groups, Bonferroni Correction was performed to adjust the alpha value and $p < 0.015$ was considered statistically significant.

Results

Total B Cells and Antibody-Secreting Cells are Increased in the Peripheral Blood of Patients with Glaucoma

To determine the phenotypic changes of peripheral B cells in glaucoma patients. We performed flow cytometry to determine the subset distribution and functional changes of B cells (Figure 1).[36] First of all, we analyzed whether the amount of total B cells was altered. Flow cytometric staining for CD19 (gated on total live PBMC), the lineage marker of B cells, showed a significant increase of $CD19^+$ cells (total B cells) (Figure 2A and B). After antigen stimulation, B cells become activated B cells with the help of antigen-presenting cells and T helper cells, and then differentiate into plasmablasts, which are further differentiated and proliferated into antibody-secreting cells (ASC) (also called plasma cells). ASC are able to synthesize and secrete various kinds of antibodies. As showed in Figure 2C and D, $CD19^+$ $CD27^+$ $CD38^+$ cells were identified as ASC/plasmablasts and glaucoma patients were

Table 1 Demographics of the Study Groups and Clinical Characteristics of Glaucoma

Variables	Glaucoma	Healthy Donors	*P* value
n	44	36	–
Age (year)	52 ± 7	50 ± 8	0.82[a]
Age distribution			0.9783[b]
≤ 40	3 (6.8%)	2(5.6%)	
41–49	12 (27.3%)	11(30.6%)	
50–59	27 (61.4%)	21(58.3%)	
≥ 60	2 (4.5%)	2(5.6%)	
Sex, female	21 (47.7%)	17 (47.2%)	> 0.99[c]
Clinical type			
PACG/POAG	38/6	–	–
IOP, mean	29.22 ± 1.81	16.45 ± 0.21	<0.001[d]
Duration[e]			
Group 1 (day)	12.83 ± 5.15	–	–
Group 2 (month)	5.29 ± 2.56	–	–
Group 3 (year)	4.14 ± 2.88	–	–
Glaucoma severity			
Mild	17 (38.6%)	–	–
Moderate	8 (18.2%)	–	–
Severe	19 (43.2%)	–	–

Notes: Data are presented as mean ± SD when applicable. [a]Student's *t*-test (unpaired, two-tailed). [b]Chi-square test. [c]Chi-square test. [d]Student's *t*-test (unpaired, two-tailed). [e]All patients enrolled in this study were divided into 3 groups based on their disease duration: 1, duration of shorter than 1 month (n=6); 2, 1–12 months (n=17); 3, longer than 1 year (n=21).

Abbreviations: PACG, primary angle-closure glaucoma; POAG, primary open-angle glaucoma; IOP, intraocular pressure.

found to have a significant increase in the frequency and absolute number of this subset compared to HD.

Peripheral B Cell Subsets are Altered in Patients with Glaucoma

Using our approach, we segregated non-ASC/plasmablasts B cells into 4 classical subsets in the peripheral circulation based on CD27 and IgD expression: $CD19^+$ $CD27^-$ IgD^+ naïve B cells, $CD19^+$ $CD27^+$ IgD^+ unswitched memory B cells (also called IgM memory B cells), $CD19^+$ $CD27^+$ IgD^- classical switched memory B cells, and $CD19^+$ $CD27^-$ IgD^- double negative (DN) B cells (also called late memory B cells) (Figure 1).[37] We first looked at the naïve compartment and found a slight but significant increase in glaucoma patients compared to HD (Figure 3A and B). Moreover, naïve population could be additionally separated by CD24 and CD38 expression subdivided into $CD24^+$ $CD38^+$ transitional cells, $CD24^-$ $CD38^+$ mature cells, and a non-classic $CD24^+$ $CD38^{-/low}$ cells (Figure 1). We observed a significant increase in the transitional (Figure 3C and D) and $CD24^+$ $CD38^{-/low}$ (Figure 3G and H) fraction in glaucoma patients compared to HD. Although no significant changes were found in the frequency of the mature fraction (Figure 3E), the absolute number was markedly up-regulated (Figure 3F). Next, the memory compartments in both glaucoma patients and HD were checked. The unswitched memory subset was significantly reduced in the frequency (Figure 4A and B), but the absolute number of switched memory subset was enlarged in glaucoma patients compared with HD (Figure 4C and D). In addition, we observed that both frequency and absolute number of $CD27^-$ IgD^- DN B cells were evidently increased in glaucoma compared to those in HD (Figure 4E and F).

Altered Peripheral B Cell Subsets are Associated with the Clinical Severity of Glaucoma

Next, we aimed to investigate whether altered B cell subsets were associated with the clinical severity of glaucoma. We divided glaucoma patients into 3 categories based on visual field defects as described above. No significant differences in the total B cells and ASC/plasmablasts were observed among glaucoma patients with different severity (Supplementary Figure 1A and B). When B cell subsets were checked, we found that the frequency of $CD27^-$ IgD^- DN subset was significantly higher in severe cases than that in mild cases (Figure 5A), though the increase in their absolute number was insignificant as the disease severity worsened. The reduction of the unswitched memory B cells was significantly magnified according to the clinical severity (Figure 5C and D). No remarkable changes were detected of the switched memory, naïve, mature, transitional and $CD24^+$ $CD38^{-/low}$ subsets among these 3 clinical categories of patients (Supplementary Figure 1C–G). Of note, we did not find any evident alterations of peripheral B cell subsets among patients with different disease duration (Supplementary Figure 2), or between those with different clinical subtypes (Supplementary Table 1) in the current study.

Discussion

In the current study, we investigated the alterations of B cell subsets in the peripheral blood of patients with glaucoma. To our best knowledge, this is the first report

to demonstrate B cell subset alterations in human glaucoma. Our findings in glaucoma patients revealed that: 1) The number of total B cells were increased, indicative of an excessive B cell-mediated immune response in the disease. 2) ASC/plasmablasts were also up-regulated, which suggested that antibody secretion might be exaggerated. 3) Naïve B cells showed a slight but significant increase, indicating that B cell generation might be enhanced. Additionally, a significant increase was observed in the transitional ($CD24^+$ $CD38^+$) fraction. It has been demonstrated that inflammatory context facilitates the expansion of the $CD24^+$ $CD38^+$ transitional compartment in various autoimmune or infectious disorders.[38–40] Although this compartment is typically considered as "naïve", their capacity of differentiating into ASC has been reported.[41] We here speculate that this transitional population might be associated with ASC differentiation and antibody production, and contribute to the glaucoma pathogenesis, but further investigation is required. 4) As for memory B cells, a decrease in the unswitched memory subset and an increase in the switched memory subset were found in glaucoma patients, suggestive of a possibility that pre-existing memory B cells might be re-activated and differentiate into atypical late memory B cells ($CD27^-$ IgD^- DN subset, which was found to be enlarged in the disease) or effector cells, such as ASC/plasmablasts.[42–44] 5) The amount of $CD27^-$ IgD^- DN subset was positively associated with the clinical severity of glaucoma, prompting us to speculate their potential to serve as biomarkers to monitor the disease progression. Interestingly, we did not find any significant alterations among groups when all enrolled patients were divided into 3 groups based on disease duration (Supplementary Figure 2) or into 2 groups based on disease subtype (Supplementary Table 1). We do realize that there are limitations in our current study, such as the limited sample size and failure to examine B cell subsets in retina biopsies of glaucoma patients, which are urgently needed to be addressed in future research.

Evidences suggest that neuroinflammation play a critical role in the pathogenesis of glaucoma.[12] Unlike non-neural tissues, the retina is protected by the blood-retina barrier (BRB) from the disturbance from peripheral immune cells, and exhibits an "immune privilege" nature. However, several recent studies by different groups have provided compelling evidence that peripheral leukocytes might be involved in the pathogenesis of glaucoma. Studies have found that DBA/2J mice (which have spontaneous glaucoma) are largely protected from glaucomatous retinal degeneration after peripheral monocytes being removed by radiation, suggesting that the occurrence and development of glaucoma might require the participation of retinal innate immunity and peripheral immune cells.[45] Adoptive transfer of lymphocytes from glaucoma mice induces glaucomatous RGC loss in recipient mice.[15] Deficiency in T and B cells alleviates IOP elevation-induced RGC loss in mice.[46] Therefore, increasing attention has been paid to the profile and role of peripheral lymphocytes. Recent studies have shown that peripheral T cell subsets are altered in glaucoma. The frequency of regulatory T cells (Treg) was decreased within the entire $CD4^+$ or $CD8^+$ population in glaucoma.[47] Circulating IL-17A-producing $CD4^+$ T cell (known as Th17) frequency was found to be increased in patients with glaucoma.[47] Besides T cells, B cells are one of the two arms of adaptive immunity, which are derived from bone marrow pluripotent stem cells. They play a role in the humoral immune components of the adaptive immune system by secreting antibodies. Although much effort has been made to elucidate the role of antibodies in glaucoma, the role of B cells themselves in the disease remains unknown. In fact, some studies have paid attention to B cells in ocular diseases. For example, up-regulated levels of IgA and plasmablasts were observed in the peripheral blood of patients with age-related macular degeneration.[48] Moreover, plasma cell infiltration has been found in the iris of children with ana-positive anterior uveitis,[49] and regulatory B cells play an immunosuppressive role in uveitis in an IL-10/IL-35 dependent manner.[50] Additionally, it has even been considered to apply B cell depletion therapy (anti-CD20 monoclonal antibody, Rituximab) in uveitis.[51,52] As for glaucoma, no reports, to our knowledge, have explored the peripheral B cell subset alterations. Our findings reveal that peripheral B cell subset are altered in glaucoma patients, but these data also raise several follow-up questions, such as how these alterations occur, are they the etiological factors or just the consequences of the disease progression, whether and how B cells contribute to the pathogenesis of glaucoma, and functions of different B cell subsets in the disease. Previous studies by other groups have shown B cell infiltration in the retina of glaucoma patients,[24,25] and that mice deficient in B cells display reduced RGC axon loss in the prolonged phase than wild-type controls.[46] These evidences suggest a role of B cells in the development of glaucoma. Based on these observations in this study, we have already

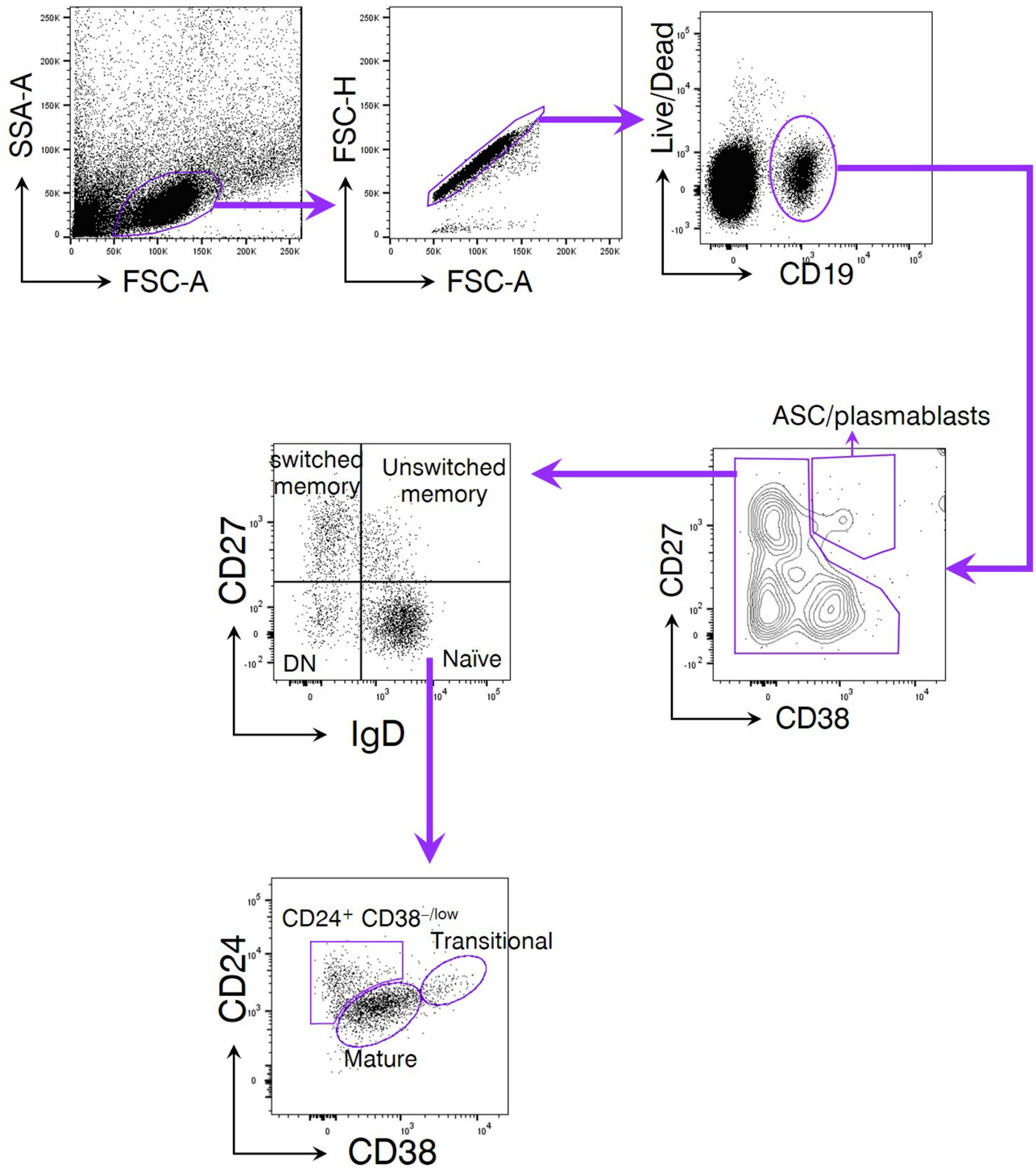


Figure 1 The gating strategy of peripheral blood B cell subsets. Peripheral blood mononuclear cells (PBMC) were isolated from subjects enrolled in the current study. A LIVE/DEAD™ Fixable Near-IR Dead Cell Stain Kit was used to determine the viability of PBMC. CD19 was used as the lineage marker of total B cells. CD27 and CD38 were coupled to discriminate antibody-secreting cells (ASC)/plasmablasts due to their high expression of both markers. Non-ASC/plasmablasts B cells were segregated into 4 classical subsets based on CD27 and IgD expression: $CD19^+$ $CD27^-$ IgD^+ naïve B cells, $CD19^+$ $CD27^+$ IgD^+ unswitched memory B cells (also called IgM memory B cells), $CD19^+$ $CD27^+$ IgD^- classical switched memory B cells, and $CD19^+$ $CD27^-$ IgD^- double negative (DN) B cells (also called late memory B cells). Among them, the naïve fraction could be additionally separated by CD24 and CD38 expression subdivided into $CD24^+$ $CD38^+$ transitional cells, $CD24^-$ $CD38^+$ mature cells, and a non-classic $CD24^+$ $CD38^{-/low}$ cells.

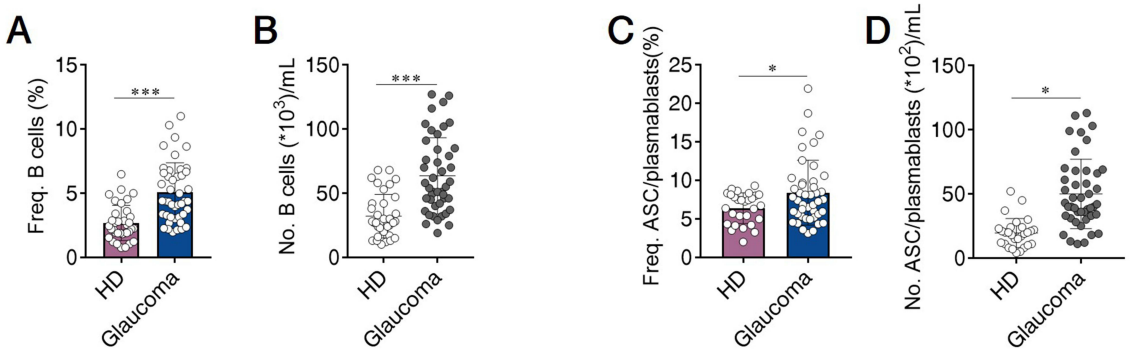


Figure 2 Total B cells and antibody-secreting cells (ASC)/plasmablasts are increased in the peripheral blood of patients with glaucoma. Peripheral blood mononuclear cells (PBMC) were isolated from patients with glaucoma (n=44) and healthy donors (HD, n=36). Total B cells and ASC/plasmablasts were identified as described in Figure 1. The frequency (Freq.) and absolute number (No.) of (**A** and **B**) total B cells and (**C** and **D**) ASC/plasmablasts in glaucoma patients and HD were shown. *p<0.05, ***p<0.001. Statistical comparisons were performed using unpaired Student's *t*-test (two-tailed). The data are represented as means ± SD.

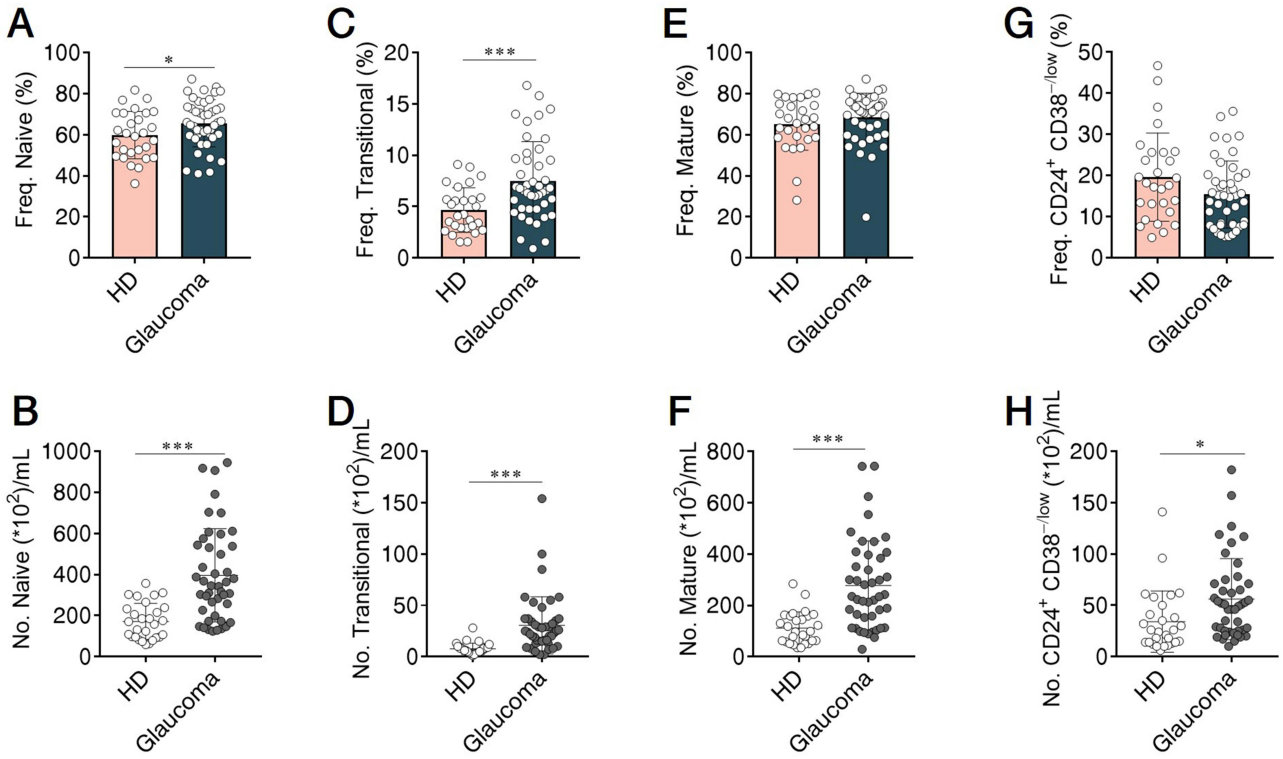


Figure 3 Altered naïve B cell compartments in patients with glaucoma. PBMC were isolated and naïve B cell compartments were gated as in Figures 1 and 2. The frequency and absolute number of (**A** and **B**) total naïve B cells, and (**C** and **D**) transitional, (**E** and **F**) mature, (**G** and **H**) $CD24^+$ $CD38^{-/low}$ subpopulations in glaucoma patients and HD were shown. *p<0.05, ***p<0.001. Statistical comparisons were performed using unpaired Student's *t*-test (two-tailed). The data are represented as means ± SD.

established IOP elevation-induced glaucoma mouse model and also find that the number of B cells is changed in the spleen, peripheral blood, and bone marrow (data not shown). Furthermore, we observe an altered chemokine profile on peripheral B cells in glaucoma mice (data not shown). Since chemokines play a crucial role in leukocyte migration and have been important targets for developing novel therapeutics in certain autoimmune diseases,[53–55] our ongoing experiments point to several exciting directions for future research and night provide useful ideas for searching therapeutic targets in glaucoma, especially for which could be targeted by drugs that have already been applied in other clinical settings.

B cells are divided into several subpopulations according to their surface markers and often divided into four subgroups by using IgD and CD27.[37] CD27, one member of the TNF-R superfamily, is a universal surface marker of memory B cells.[56,57] The expression of IgD, primarily expressed

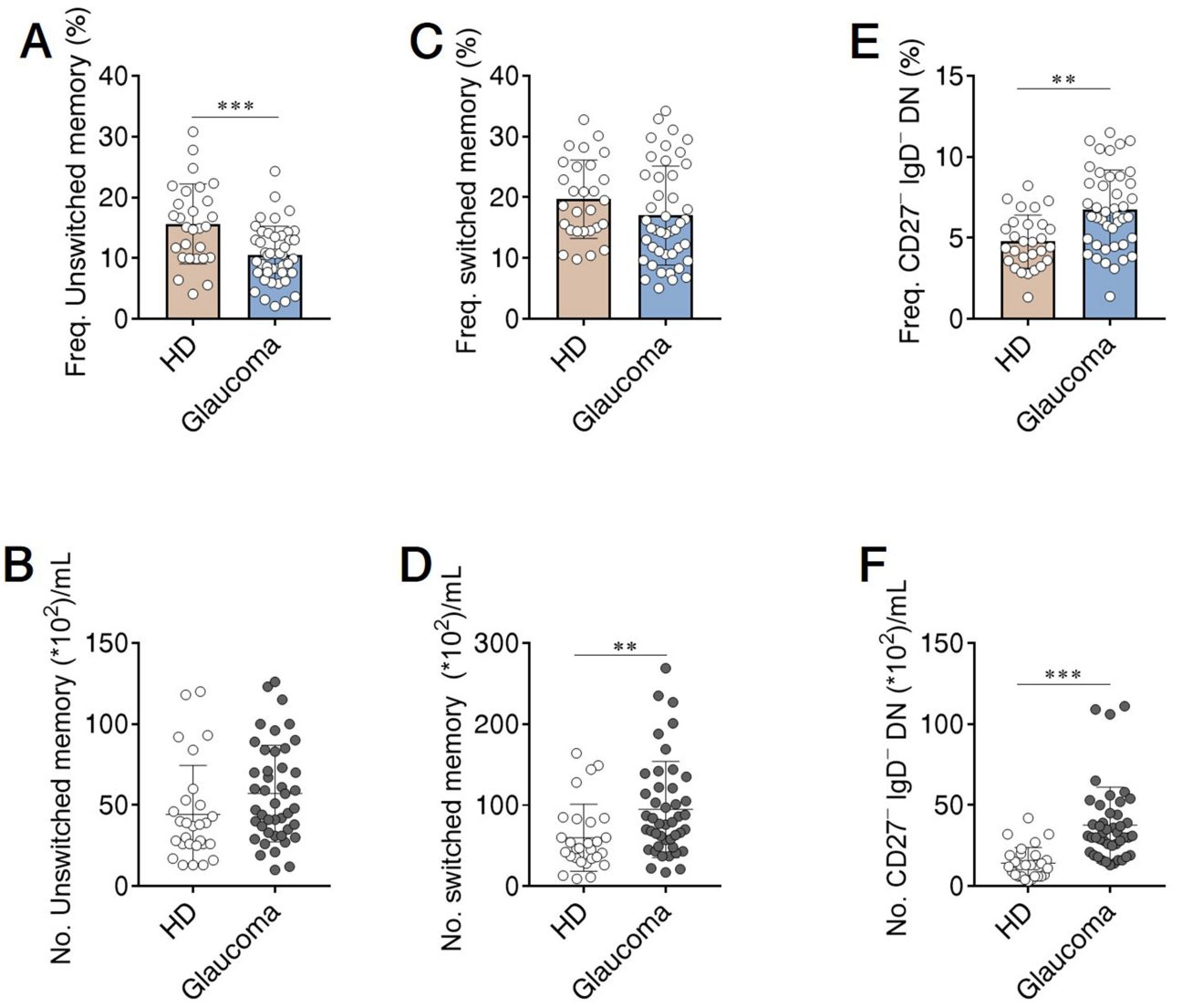


Figure 4 Memory B cell subsets were changed in patients with glaucoma. PBMC were isolated and memory B cell compartments were gated as in Figures 1 and 2. The frequency and absolute number of (**A** and **B**) unswitched memory, (**C** and **D**) switched memory, and (**E** and **F**) $CD27^-$ IgD^- DN subsets in glaucoma patients and HD were shown. **p<0.01, ***p<0.001. Statistical comparisons were performed using unpaired Student's *t*-test (two-tailed). The data are represented as means ± SD.

by naïve B cells, is decreased when B cells have undergone isotype switch. Due to the absence of CD27, DN and naïve B cells were previously indistinguishable. On the other hand, the low expression of IgD indicates that DN B cells might be activated after antigen stimulation.[58,59] So paradoxically, DN B cells, unlike the other three subgroups, have been poorly understood for decades. However, studies on this subset have been increasing in the last few years, providing evidences to improve our knowledge on their functions in immune responses. DN B cells are now widely accepted as a novel subset of memory rather than naïve B cells. Under normal physiological conditions, DN B cells can be found in the frequency of approximately 5% of all peripheral blood B cells and the rate becomes higher in the elderly.[60–64] DN B cells have recently emerged in many inflammatory and aging diseases, particularly in autoimmune disorders. For example, $CD27^-$ IgD^- B cell proportion correlates with the clinical manifestations,[65,66] and upregulation of autoantibodies of SLE patients.[65,67] Mechanistically, $CD27^-$ IgD^- B cells are able to become plasmablasts and plasma cells and subsequently secrete autoantibodies.[66,68] Our current study shows that $CD27^-$ IgD^- DN B cells are increased and positively correlated with glaucomatous visual field loss, indicative of a pathogenic role of this B cell subset.

Although future in-depth studies are urgently needed to address issues such as the roles of different B cell subsets in glaucoma, how $CD27^-$ IgD^- B cells contribute to the

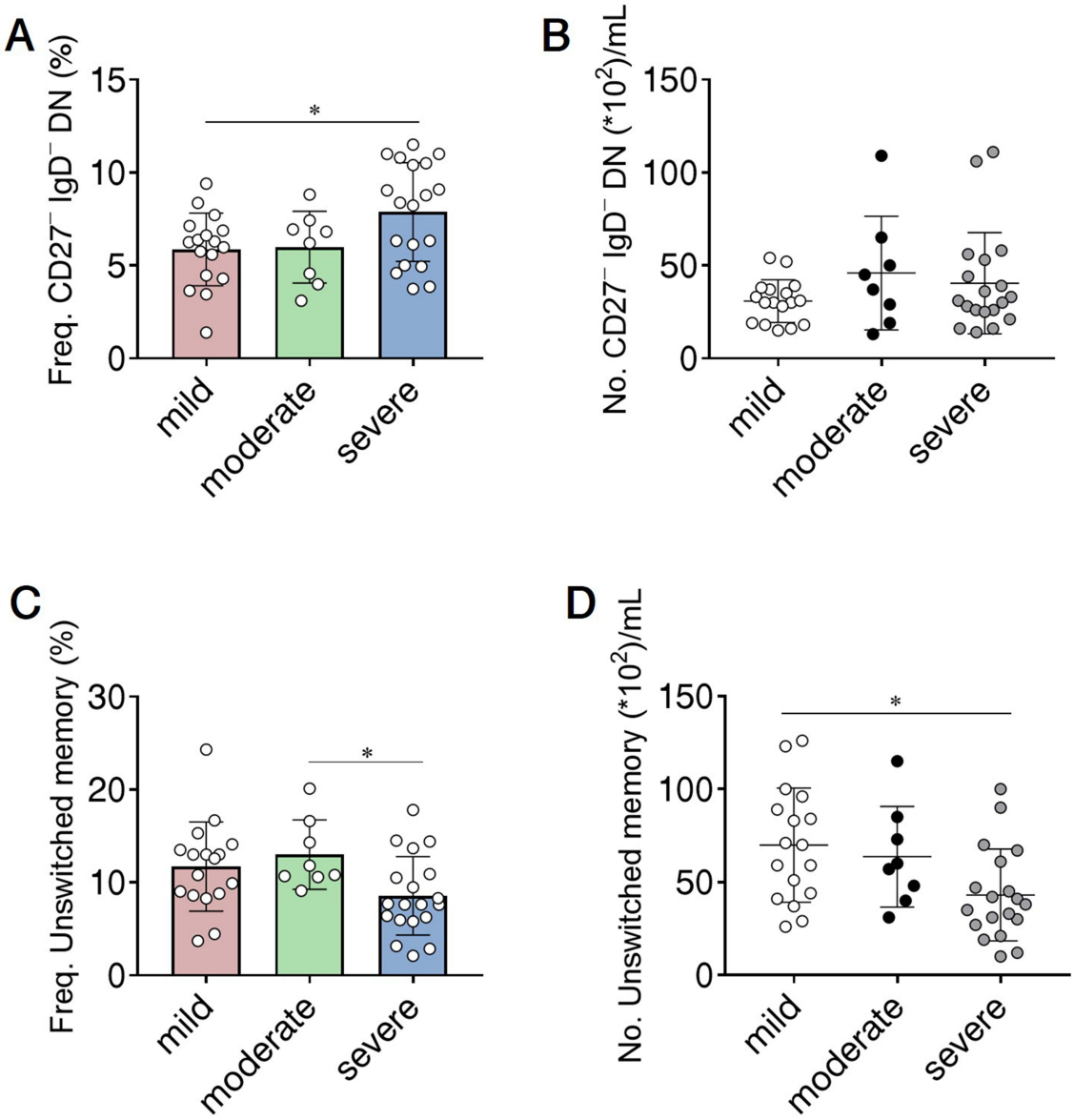


Figure 5 B cell subsets correlate with the clinical severity of glaucoma. The severity of glaucoma was determined based on the mean deviation (MD) of visual field: mild indicates visual field MD of greater than −6 dB (n=17); moderate, −12 dB to −6 dB (n=8); and severe, no greater than −12 dB (n=19). The frequency and absolute number of (**A** and **B**) $CD27^-$ IgD^- DN and (**C** and **D**) unswitched memory subpopulations in 3 clinical categories of glaucoma patients. *p<0.0167, one-way analysis of variance (ANOVA) was applied followed by Bonferroni Correction. The data are represented as means ± SD.

pathogenesis, and how their differentiation and function are regulated, our study reveals the clinical severity of glaucoma is accompanied by alterations in the B cell subsets, and the correlation between B cell subsets and glaucoma severity suggests that they might be able to serve as potential biomarkers to monitor the disease progression.

Abbreviations

MGC, microglial cells; IL, interleukin; TNF, tumour necrosis factor; PBS, phosphate buffered saline; RGC, retinal ganglion cells; ON, optic nerve; NTG, normal tension glaucoma; PACG, primary angle-closure glaucoma; POAG, primary open-angle glaucoma; ASC, antibody-secreting cells; IOP, intraocular pressure; DN, double negative; MD, mean deviation; PBMC, peripheral blood mononuclear cells; HD, healthy donors; Ig, immunoglobulin; BSA, bovine serum albumin; SD, standard deviation.

Ethics Approval

The study was conducted in accordance with the Declaration of Helsinki and approved by the Institutional Review Board for Clinical Research of the Daping

Hospital (No. 202192), and Sichuan Provincial People's Hospital (No. 201968). All subjects were well informed about the study and potential risk and signed an informed consent before participation.

Funding

This work is financially supported by grants from the National Natural Science Foundation of China (U19A2004, 81970803, 82070985) and Foundation of Sichuan Science and Technology Department (2019JDTD0014, 2021JDJQ0044).

Disclosure

The authors declare no competing interests.

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