· Basic Research ·

Neuroprotectin D1 regulating the inflammation and nerve restoration in *Aspergillus fumigatus* keratitis

He Li, Yi–Yi Jia, Meng–Qi Wu, Li–Na Zhang, Jing Lin, Qian Wang, Cui Li, Gui–Qiu Zhao

Department of Ophthalmology, The Affiliated Hospital of Qingdao University, Qingdao 266003, Shandong Province, China
Correspondence to: Cui Li and Gui-Qiu Zhao. Department of Ophthalmology, The Affiliated Hospital of Qingdao University, Qingdao 266003, Shandong Province, China. yankelicui@126.com; zhaoguiqiu_good@126.com
Received: 2025-11-03 Accepted: 2026-02-04

Abstract
● **AIM:** To investigate the anti-inflammatory and neurorestorative effects of neuroprotectin D1 (NPD1) in *Aspergillus fumigatus* (*A. fumigatus*) keratitis in C57BL/6 mice.
● **METHODS:** The left corneas of C57BL/6 mice were infected with *A. fumigatus*. Each mouse was injected intraperitoneally with 2 μg NPD1 at 1, 3, and 5d post infection (p.i.) and injected subconjunctivally with 5 μL NPD1 once a day. The severity of keratitis was observed by slit lamp and classified by clinical score at 1, 3, and 5d p.i. Hematoxylin and eosin (HE) staining was used to evaluate the histological changes and inflammatory cell infiltration of corneas at 3 and 5d p.i. Polymerase chain reaction (PCR), enzyme-linked immunosorbent assay (ELISA), and Western blot were performed to detect the expression of IL-1β, MIP-2, CGRP, p-p38/p38 MAPK, Nrf-2, and HO-1. Immunofluorescence staining was utilized to assess the neutrophil infiltration and neural innervation. Flow cytometry assay was performed to test the number of total, M1 and M2 macrophages.
● **RESULTS:** In *A. fumigatus* infected C57BL/6 mice, NPD1 treatment ameliorated the severity of keratitis. Compared with the phosphate-buffered saline (PBS) control group, NPD1 treatment inhibited the infiltration of neutrophils and macrophages, increased the ratio of $CD206^+/CD86^+$ macrophages, reduced the phosphorylation level of p38 MAPK, and enhanced the expression of Nrf-2/HO-1 at 3d p.i. The number of corneal nerves and the mechanical sensitivity threshold of the NPD1 treatment group were significantly higher than the PBS group at 3 and 5d p.i. Meanwhile, the expression of IL-1β and MIP-2 in the NPD1 treatment group was significantly lower at 3 and 5d p.i.
● **CONCLUSION:** NPD1 improves the prognosis of *A. fumigatus* keratitis in mice by inhibiting the recruitment of neutrophils and macrophages, promoting M2 macrophage polarization and corneal nerve regeneration.
● **KEYWORDS:** neuroprotectin D1; *Aspergillus fumigatus* keratitis; inflammation; corneal nerve; macrophages; neutrophils
DOI:10.18240/ijo.2026.05.01

Citation: Li H, Jia YY, Wu MQ, Zhang LN, Lin J, Wang Q, Li C, Zhao GQ. Neuroprotectin D1 regulating the inflammation and nerve restoration in *Aspergillus fumigatus* keratitis. *Int J Ophthalmol* 2026;19(5):837-845

INTRODUCTION
Fungal keratitis is a highly destructive corneal infection. Fungal keratitis has both high incidence (approximately 1 000 000 corneas/year) and high blindness rate among infectious keratitis. It often results in permanent blindness[1-2]. Although the corneal pathogenic fungi can be classified into more than 100 different species, over 95% of them are caused by filamentous fungi *Fusarium, Aspergillus* and yeast *Candida spp.*[3-4]. Corneal infections which were caused by filamentous fungi usually have a worse prognosis than those which were caused by yeast[5]. However, there are still no effective therapeutic drugs and methods for fungal keratitis. Inflammation and nerve loss were the important manifestations in fungal keratitis[6-7]. The excessive inflammatory response is absolutely an important cause of corneal blindness in fungal keratitis[7-9]. Corneal nerve injury also makes a decrease in both epithelial stem cell function and corneal response activity to external injury, and results in an ulcer enlargement or delayed healing[10-11]. Thus, effective repairing nerve and anti-inflammatory therapy can restore ocular surface homeostasis, improve the efficacy and prognosis of patients and reduce the rate of blindness.

Neuroprotectin D1 (NPD1) is the first neuroprotective medium identified from docosahexaenoic acid (DHA)[12-15]. DHA is an ω-3 polyunsaturated fatty acid, which is mainly synthesized from dietary essential omega-3 linolenic acid in the liver and concentrated in the phospholipids of cell membranes through

the blood circulation[16-17]. NPD1 is an important mediator to exert the protective biological activity of DHA, which was originally found in oxidative stress threatened nerves[18] and retinal pigment epithelial cells[19]. It has been confirmed that NPD1 holds anti-inflammatory effects and it can exert an anti-inflammatory effect in acute inflammation[12,20-22]. A previous study has indicated that NPD1, which was induced by the pigment epithelium-derived factor (PEDF) of injured corneal epithelium, inhibited the production of the leukotriene B4 and promoted nerve regeneration in rabbit corneas[23]. As a neuroprotective medium, NPD1 has also been reported to recover the integrity of nerves in stromal dissected rabbit corneas[24].

To date, it is not clear whether NPD1 has therapeutic effects on *Aspergillus fumigatus* (*A. fumigatus*) keratitis. The purpose of this paper is to determine whether NPD1 can play a protective role in mice *A. fumigatus* keratitis by regulating the inflammation and nerve restoration.

MATERIALS AND METHODS

Ethical Approval The study was approved by the Research Ethics Committee of the Affiliated Hospital of Qingdao University (Approval No.QYFY WZLL 42022).

Production of *A. fumigatus* Hyphae Number 3.0772 *A. fumigatus* stain came from China General Microbiological Culture Collection Center (Beijing, China) and was planted in Sabouroud mediums. After a 2-3d incubation at 37℃ with shaking, the hyphae were taken out and grind evenly. Finally, the grated hyphae were diluted to 3×10^8 colony-forming units (CFU)/mL with DMEM.

Mice Models of Fungal Keratitis All operations performed on 8-week-old female C57BL/6 mice (SiPeiFu Biotechnology, Beijing, China) and followed the Statement about the Use of Animals in Ophthalmology and Vision Research declared by ARVO. Before operation, all mice were anesthetized by chloral hydrate and placed under a microscope. Then the central corneal epithelium, 2 mm approximately in diameter, was removed in the left eye. Eyelids was sutured after coating 5 μL *A. fumigatus* hyphae and covering with a soft contact lens. At next day (24h), remove stitches and take out the lens. Then mice received treatments combined 2 μg NPD1 or phosphate-buffered saline (PBS; 7% alcohol) peritoneal injection at 1, 3, and 5d post infection (p.i.) and 5 μL NPD1 or PBS subconjunctival injection every day. Clinical score was performed to assess the degree of fungal keratitis at 1, 3, and 5d p.i[25]. In short, accounted the corneal opacity area, opacity density, and regularity of corneal surface on a scale of 1-4 points, respectively. The more severe the performance, the higher the score. Then sum score of all sections is corneal clinical score. Then mice corneas or whole eyeballs were harvested and used for follow-up experiments.

Von Frey Test The corneal mechanical sensitivity thresholds were examined by the Von Frey threshold test by the up-down method. In short, mice were gently held by surgical towels and the corneas were exposed completely in a stereoscopic microscope. A range of Von Frey fibers (Stoelting Co., IL, USA) were used to touch the surrounding areas of corneal ulcer, and data of fibers was recorded when the blink response was observed in mice. To accurately measure mechanical sensitivity, each cornea is repeatedly stimulated five times.

Real-time Polymerase Chain Reaction Polymerase chain reaction (PCR) was utilized to test mRNA expression of mice corneas. The protocol was the same as our previous study[26]. Corneas (*n*=6/group) were collected and placed each cornea in 800 μL RNAiso Plus reagent (Takara, Dalian, China). Total RNA was extracted and concentration was measured. Then 2 μg total RNA system was configurated. Next, RNA was reverse-transcribed to cDNA according to the manual of the HiScript III RT SuperMix for qPCR (+gDNA wiper; Vazyme, China). PCR was finally performed and test by Roche LightCycler 96. Nucleotide sequences of mouse primers for PCR are listed in Table 1.

Western Blot Western blot (WB) was performed to evaluate the degree of phosphorylation of p38 mitogen-activated protein kinase (p38 MAPK) as well as the expression of calcitonin gene-related peptide (CGRP) and nuclear factor erythroid 2-related factor 2 (Nrf-2)/heme oxygenase-1 (HO-1). All mice corneas in each group (*n*=6/group) were gathered together and placed in a 200 μL lysate (1% phosphatase inhibitor cocktail and 1% PMSF, Solarbio). After 2h decomposition in the ice, centrifuge the sample (4℃, 12 000 rpm, 5min) and collect the supernatant to measure concentration of total protein with the bicinchoninic acid (BCA) protein assay kit (Solarbio). The 40 μg protein sample was used and electrically dispersed to the sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), and then transferred to polyvinylidene difluoride membrane (PVDF membrane, Solarbio). The protein-loaded PVDF membrane was blocked with blocking buffer (Beyotime Biotechnology, China) for 2h, and then they were soaked in relative primary antibodies at 4℃ overnight, which contain Nrf2 (1:1000; Abcam, Cambridge, UK), HO-1 (1:1000; Abcam), p-p38 (1:1000, CST), p38 (1:1000, CST), CGRP (1:1000, CST), β-tubulin (1:2000; Elabscience) and glyceraldehyde-3-phosphate dehydrogenase (GAPDH; 1:2000; Elabscience). Next day, the PVDF membranes were further immersed in the secondary antibodies (1:2000; Elabscience) for 1h at 37℃ followed by detected with enhanced chemiluminescence (ECL, Vazyme). Image J software was used to quantify the band intensities. The relative protein expression levels were normalized to GAPDH or β-tubulin as internal controls.

Table 1 Primer list used for PCR

Gene (mouse)	Nucleotide sequence	Primer	Gen bank
β-actin	5'-GATTACTGCTCTGGCTCCTAGC-3'	F	NM_007393.3
	5'-GACTCATCGTACTCCTGCTTGC-3'	R	
IL-1β	5'-CGCAGCAGCACATCAACAAGAGC-3'	F	NM_008361.4
	5'-TGTCCTCATCCTGGAAGGTCCACG-3'	R	
MIP-2	5'-TGTCAATGCCTGAAGACCCTGCC-3'	F	NM_009140.2
	5'-AACTTTTTGACCGCCCTTGAGAGTGG-3'	R	
CGRP	5'-GGACTTGGAGACAAACCACCA-3'	F	NM_007587.2
	5'-GAGAGCAACCAGAGAGGAACTACA-3'	R	

PCR: Polymerase chain reaction; *IL-1β*: Interleukin-1β; *MIP-2*: Macrophage inflammatory protein-2; *CGRP*: Calcitonin gene-related peptide; F: Forward; R: Reverse.

Enzyme-Linked Immunosorbent Assay Enzyme-linked immunosorbent assay (ELISA) was utilized to test the corneal protein expression of inflammatory factor. Every 2 corneas were ground together and ground mechanically in 1000 μL PBS (*n*=6/group), and then they were centrifuged for supernatants (4℃, 5000×g, 10min). Detection steps were carried out completely according to the instructions of R&D ELISA kit and measured the absorbance at 450 nm and 570 nm.

MPO Assay The myeloperoxidase (MPO) activity was performed to detect the discrepancy of the number of corneal neutrophils between the NPD1 and PBS group. Corneas (*n*=6/group) were ground at 3d p.i., then subsequent experimental steps refer entirely to the manual of the MPO kit (Nanjing Jiancheng Bioengineering Institute, China).

HE Staining Corneal hematoxylin and eosin (HE) staining was performed to assess the degree of cell infiltration and tissue integrity of infected corneas. The infected eyeballs (*n*=3/group) were wholly harvested at 3 and 5d p.i. After fixed in paraformaldehyde (4%) for 3d at room temperature, the lenses were removed and eyeballs were embedded into the paraffin. Six micron slices were prepared for staining. The conventional HE staining method was performed and the slices were taken pictures by microscopes (magnification×400).

Flow Cytometry Flow Cytometry was used to test the number of corneal macrophages. The methods were consistent with the previous experiments[27]. Simply put, samples were collected at 3d p.i. Each cornea is clipped and placed in liberase (100 μL, 0.04 mg/mL, Roche, Basel, Switzerland). Then they were heated at 37℃ for 60min with shaking every 15min. After filtering, the cell suspension was incubated with CD16/CD32 FC Block solution (BD Pharmingen, Franklin Lake, NJ, USA) for 15min and CD45-PerCP, F4/80-PE-CY7, CD86-PE, and CD206-FITC (1:200; Biolegend, San Diego, CA, USA) for 20min at 4℃ successively. Finally, sample were detected by flow cytometry (Beckman Coulter, Krefeld, Germany), and the data of samples were analyzed by the FlowJo V10 software. All the experiments were repeated 3 times.

Immunofluorescence Staining Immunofluorescence staining (IFS) was used to evaluate the degree of neutrophil infiltration and corneal nerve density respectively. The method of the former was the same as previous study[28]. In short, mice eyeballs (*n*=3/group) were wholly socked in the optimal cutting temperature compound (OCT) followed by froze with liquid nitrogen at 3d p.i. Then, 10 μm corneal slices was produced and socked in acetone for 30min. After blocking, corneal slices were successively incubated with neutrophil marker antibody NIMP-R14 (1:200; Santa Cruz, USA) overnight. In the next day, slices were treated with secondary antibody (FITC-conjugated; 1:200; Elabscience) for 1h and with 4'6-diamidino-2-phenylindole (DAPI; 10 μg/mL; Solarbio) for 10min. Finally, corneal slices were taken pictures by a fluorescence microscope.

For detecting the density of the corneal nerve, the whole eyeball was collected and soaked in 1.3% paraformaldehyde-PBS solution at room temperature for 1h at 3 and 5d p.i. Then the corneas were completely exfoliated in sterile PBS and four radioactive incisions were made in the outer region of the cornea to allow the cornea to be laid flat. After washed in PBS five times (five minutes per time), corneas were treated with 1% Triton X-100 for 1h and followed by washing in PBS five times. The corneas were next placed in 20% goat serum for 1h for blocking. After that, corneas were incubated with 3 μg/mL primary anti-beta III tubulin antibody (Abcam) for 2h at room temperature and then overnight at 4℃. On the next day, corneas underwent 2h incubation with anti-rabbit secondary antibody (1:100; Elabscience) and 15min incubation with DAPI (ready-to-use; Solarbio), respectively. Finally, the corneas were fixed on the slides and captured with a fluorescence confocal microscope.

Statistical Analysis All experimental data were showed in format of mean±standard deviation (SD). GraphPad Prism 8 software was used for data visualization. The Mann-Whitney *U* test was used to evaluate the discrepancy in clinical scores between two group. An unpaired, two-tailed student's *t*-test

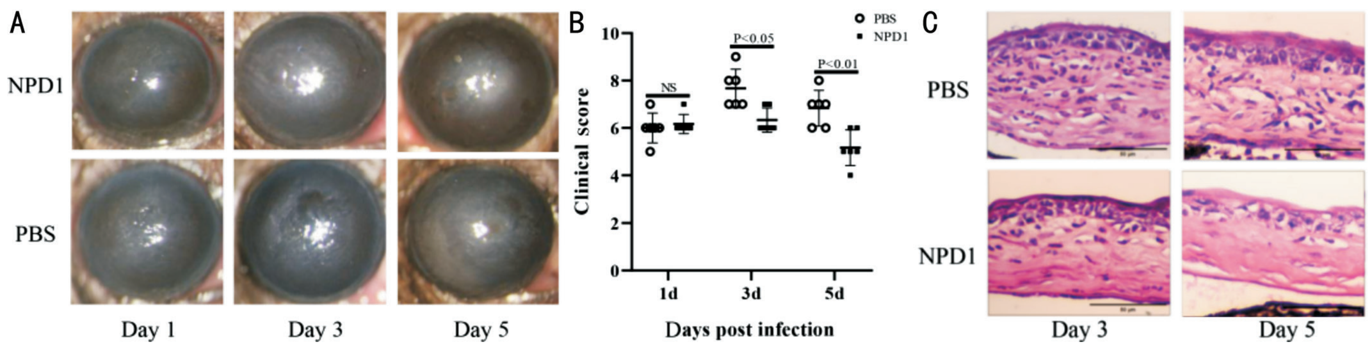

Figure 1 Effect of NPD1 in C57BL/6 mouse *A. fumigatus* keratitis A: Representative slit lamp photographs of PBS or NPD1 treated *A. fumigatus* keratitis mice cornea at days 1, 3, and 5 p.i.; B: Clinical scores of PBS or NPD1 treated mice cornea (*n*=6 mice/group); C: HE staining of corneal tissue sections of PBS or NPD1 treated *A. fumigatus* keratitis mice at 3 and 5d p.i. (*n*=3 mice/group). Magnification: 400×. All data were mean±SD and analyzed by the Mann-Whitney *U* test. NPD1: Neuroprotectin D1; PBS: Phosphate-buffered saline; *A. fumigatus*: *Aspergillus fumigatus*; p.i.: Post-infection; HE: Hematoxylin and eosin; SD: Standard deviation; NS: Not significant.

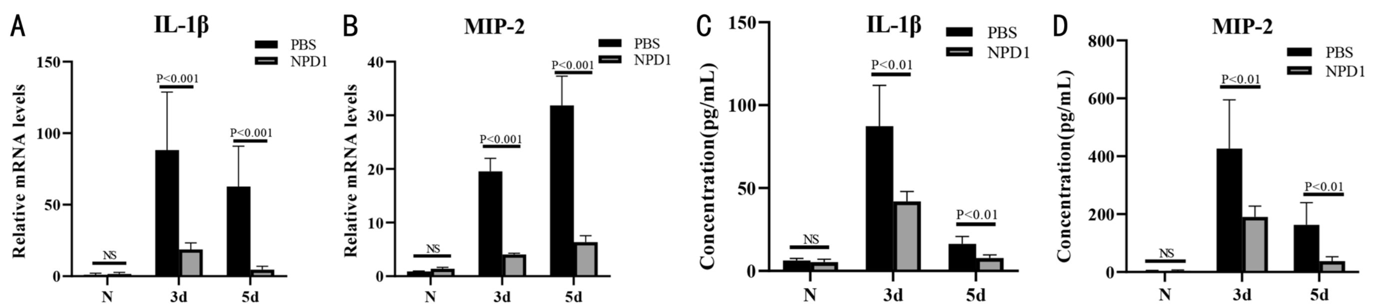

Figure 2 Anti-inflammatory activity of NPD1 in C57BL/6 mouse *A. fumigatus* keratitis PCR results for IL-1β (A), MIP-2 (B) at 3 and 5d p.i.; ELISA results of IL-1β (C), MIP-2 (D) at 3 and 5d p.i. (*n*=6 mice/group). All data were mean±SD and analyzed by an unpaired, two-tailed Student's *t*-test. NPD1: Neuroprotectin D1; *A. fumigatus*: *Aspergillus fumigatus*; PCR: Polymerase chain reaction; IL-1β: Interleukin-1β; MIP-2: Macrophage inflammatory protein-2; p.i.: Post-infection; ELISA: Enzyme-linked immunosorbent assay; SD: Standard deviation; NS: Not significant.

was performed in the data of PCR, WB, ELISA, plate count, and IFS. It was considered as significant when $P<0.05$ (ns=no significance). All experiments were repeated at least three times.

RESULTS

NPD1 Treatment Reduced the Severity of Fungal Keratitis in Mice To examine the role of NPD1 in mouse fungal keratitis, we photographed and scored the infected corneas of mice at 1, 3, and 5d p.i. Results showed that there was no significant difference in corneal clinical scores (Figure 1B; $P>0.05$) and corneal photographs (Figure 1A) between the two groups of mice at 1d p.i. However, the degree of corneal inflammation infiltration and ulceration of NPD1 treatment group was significantly lower than that of PBS group at 3 and 5d p.i. (Figure 1A), and the clinical scores (Figure 1B; $P<0.05$, $P<0.01$, respectively) showed that corneas of NPD1 treatment group significantly got lower scores than those in PBS group. Corneal HE staining (Figure 1C) was performed at 3 and 5d p.i. Results showed that the degree of corneal edema and inflammatory cell infiltration of corneas in NPD1 treatment group were significantly lighter than those in PBS group.

NPD1 Treatment Reduces the Inflammatory Response in the Cornea of Mice PCR and ELISA were utilized to test the expression of inflammatory factors in *A. fumigatus* infected corneas at 3 and 5d p.i. PCR results showed that the mRNA expression of interleukin-1β (IL-1β; Figure 2A; $P<0.001$) and macrophage inflammatory protein-2 (MIP-2; Figure 2B; $P<0.001$) of corneas in NPD1 treatment group were significantly lower than those in PBS group. ELISA results showed that the protein expression of IL-1β (Figure 2C; $P<0.01$) and MIP-2 (Figure 2D; $P<0.01$) of corneas in NPD1 treatment group were significantly lower than those in PBS group. WB was utilized to detect the changes in pro- and anti-inflammatory pathways in the cornea of mice at 3d p.i., and the results showed that compared with PBS group, the ratio of p-p38/p38 MAPK (Figure 3D, 3E; $P<0.001$) in the cornea of mice in NPD1 group was significantly reduced, and the protein expression of anti-inflammatory signaling pathway proteins Nrf-2 (Figure 3A, 3B; $P<0.001$) and HO-1 (Figure 3A, 3C; $P<0.01$) was increased.

NPD1 Treatment Reduces Inflammatory Cell Infiltration of the Cornea IFS and MPO were utilized to detect the infiltration of neutrophils. The results showed that compared to PBS group, there was a weaker fluorescence message of neutrophils (Figure 4A, 4B; $P<0.05$) and a lower MPO enzyme

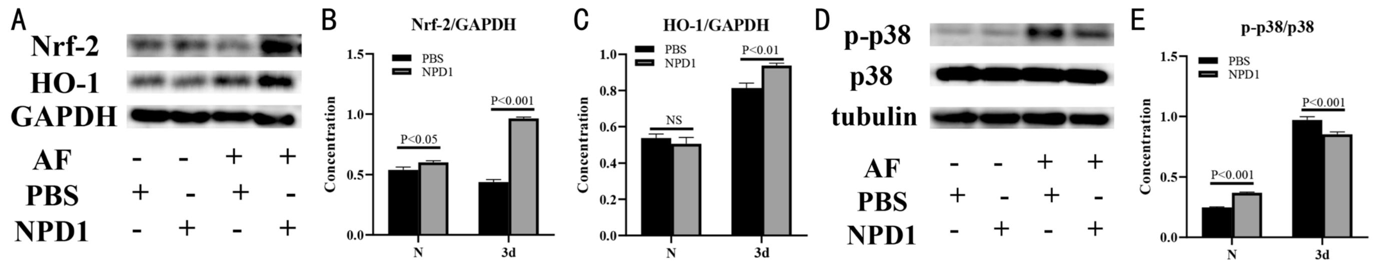

Figure 3 Effects of NPD1 on the anti- and pro-inflammatory pathway in *A. fumigatus* keratitis mouse model WB results of protein expression of Nrf-2/HO-1 pathway (A), and their grayscale value analysis, respectively (B-C) at 3d p.i. (*n*=6 mice/group). WB results of protein expression of p-p38/p38 MAPK pathway (D), and its grayscale value analysis, respectively (E) at 3d p.i. (*n*=6 mice/group). All data were mean±SD and analyzed by an unpaired, two-tailed Student's *t*-test. NPD1: Neuroprotectin D1; *A. fumigatus*: *Aspergillus fumigatus*; WB: Western blot; Nrf-2: Nuclear factor erythroid 2-related factor 2; HO-1: Heme oxygenase-1; p-p38: phosphorylated p38; p38 MAPK: p38 mitogen-activated protein kinase; p.i.: Post-infection; SD: Standard deviation; GAPDH: Glyceraldehyde-3-phosphate dehydrogenase; AF: *A. fumjgatus*; PBS: Phosphate-buffered saline; NS: Not significant.

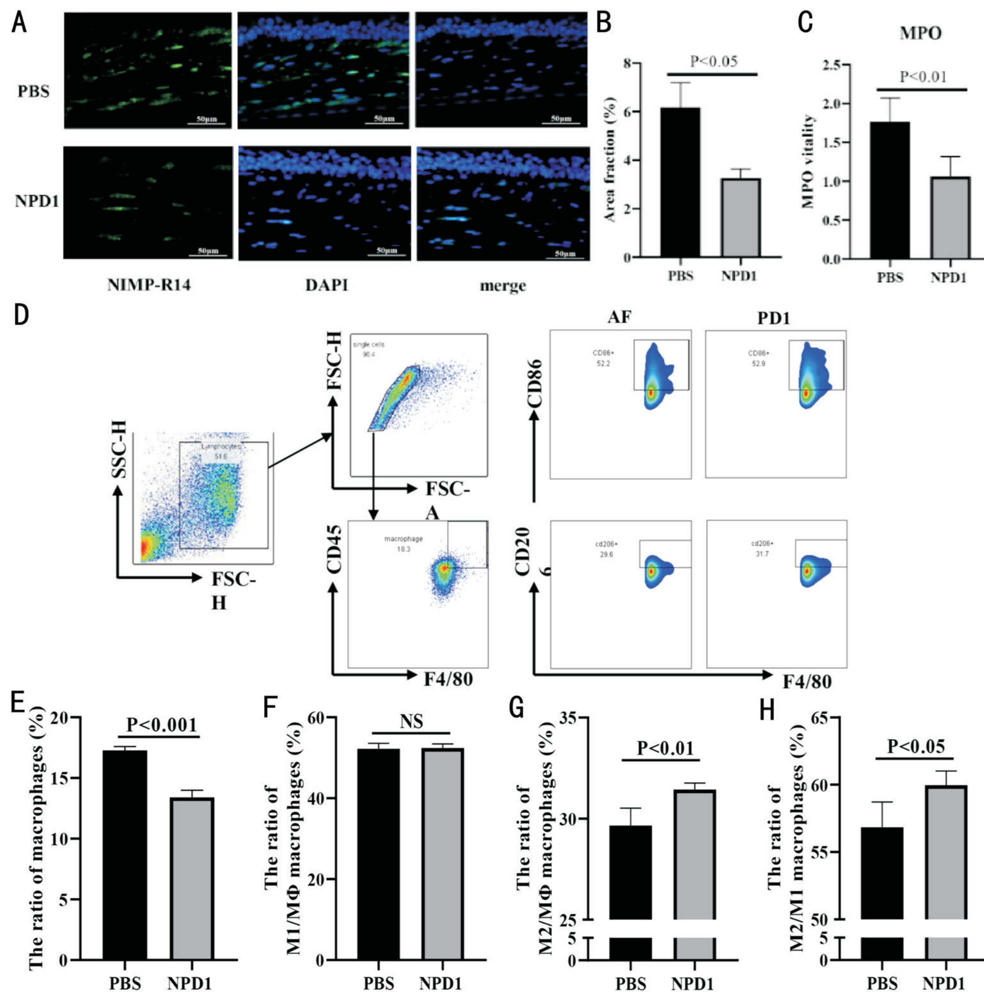

Figure 4 Effects of NPD1 on inflammatory cells infiltration in *A. fumigatus* infected C57BL/6 mice corneas Representative IFS images of infected mice corneas with NPD1 or PBS treated for accumulated neutrophils (A, green) and nucleus (A, blue), and its quantitative analysis (B; *n*=3 mice/group). MPO activity (C) in infected mice corneas with NPD1 or PBS treated (*n*=6 mice/group). Flow cytometry gating strategies (D) of M1 macrophages (CD45+F4/80+CD86+) and M2 macrophages (CD45+F4/80+CD206+). The ratio of total macrophages (E), M1/MΦ (F), M2/MΦ (G) and M2/M1 (H). Magnification: 400×. All data were mean±SD and analyzed by an unpaired, two-tailed Student's *t*-test. NPD1: Neuroprotectin D1; *A. fumigatus*: *Aspergillus fumigatus*; IFS: Immunofluorescence staining; PBS: Phosphate-buffered saline; MPO: Myeloperoxidase; SD: Standard deviation; DAPI: 4'6-diamidino-2-phenylindole; AF: *A. fumjgatus*; NS: Not significant.

activity (Figure 4C; $P<0.01$) in corneas of NPD1 treated group at 3d p.i., which indicated NPD1 treatment reduced neutrophils infiltration in corneal stroma. Flow cytometry was utilized to detect macrophages and their phenotypes in the cornea.

Results showed that compared with PBS group, the total amount of macrophages (Figure 4E; $P<0.001$) infiltrated in the cornea of mice of NPD1 group were significantly reduced. The proportion of M2 macrophages (Figure 4G; $P<0.01$) and the ratio of M2/M1 (Figure 4H; $P<0.05$) in the cornea of mice of NPD1 group was significantly higher than that of PBS group although the proportion of M1 macrophages (Figure 4F; $P>0.05$) between two groups were not significantly different.

NPD1 Treatment Promotes Corneal Nerve Repair The whole cornea was taken for IFS to detect the corneal nerves, and the results showed that both NPD1 and PBS treated group got a weak fluorescence message of the corneal nerve at 3d p.i. However, compared to PBS group, there was a stronger fluorescence message of the corneal nerve (Figure 5A, 5B; $P<0.01$) in corneas of NPD1 treated group. At 5d p.i., both NPD1 and PBS treated group got a little stronger fluorescence message of corneal nerve than those at 3d p.i., but the fluorescence message of the corneal nerve (Figure 5A, 5B; $P<0.01$) in corneas of NPD1 treated group was also stronger than that in PBS treated group. Those indicated that corneas of NPD1 treated group possessed more nerve than PBS treated group after infected with *A. fumigatus*. Von Frey Test showed that compared to the PBS treated group, the von Frey filament force of NPD1 treated group was reduced at 3 and 5d p.i. (Figure 5C; $P<0.05$), which indicated that corneas of NPD1 treated group maintain a higher sensitivity than those of PBS treated group. PCR and WB were utilized to detect the expression of CGRP, and results showed that compared with PBS group, the mRNA (Figure 5D; $P<0.05$) and protein (Figure 5E, 5F; $P<0.001$) expression of CGRP of corneas in NPD1 treatment group were significantly increased.

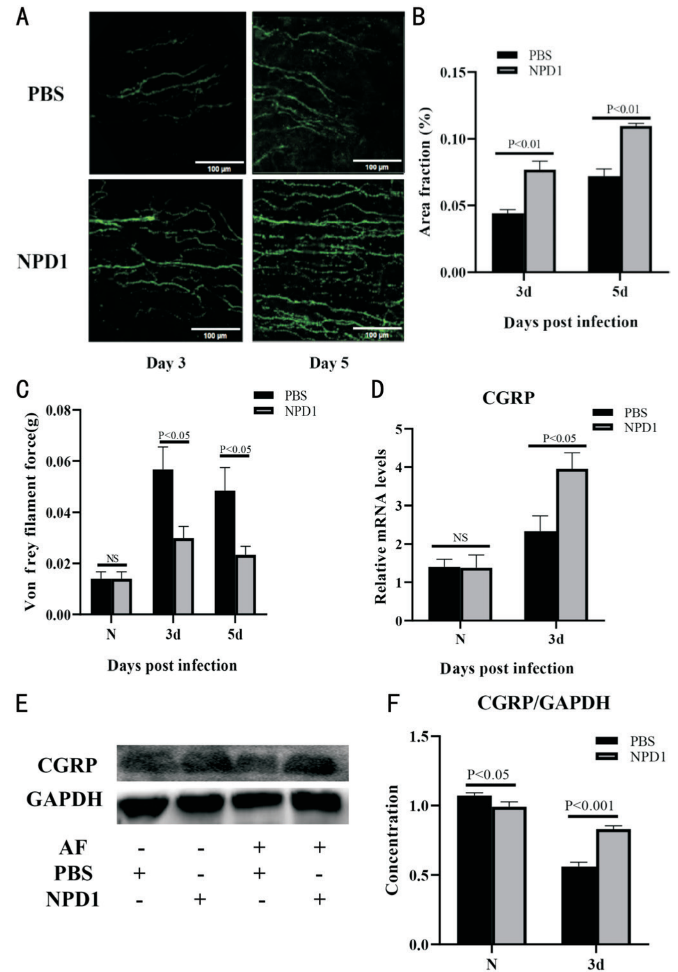

Figure 5 Nerve restoration activity of NPD1 in *A. fumigatus* infected C57BL/6 mice corneas Representative IFS images of infected mice corneas with NPD1 or PBS treated for corneal nerve (A, green) and quantitative analysis (B; n=3 mice/group). Von Frey test of infected mice corneas with NPD1 or PBS treated for mechanical sensitivity thresholds (C). mRNA (D) and protein (E, F) expression of CGRP. All data were mean±SD and analyzed by an unpaired, two-tailed Student's *t*-test. NPD1: Neuroprotectin D1; *A. fumigatus*: *Aspergillus fumigatus*; IFS: Immunofluorescence staining; PBS: Phosphate-buffered saline; CGRP: Calcitonin gene-related peptide; SD: Standard deviation; GAPDH: Glyceraldehyde-3-phosphate dehydrogenase; AF: *A. fumjgatus*; NS: Not significant.

DISCUSSION

Fungal keratitis is a refractory infectious corneal disease with a high rate of blindness. In the course of fungal keratitis, the corneal inflammatory response is not only the resistance to pathogenic fungi but also the destroyer of corneal tissue structure. The excessive inflammatory response will aggravate tissue damage in fungal keratitis, which can lead to corneal ulceration, perforation, blindness and even eyeball loss[21,29]. Thus, controlling excessive corneal inflammatory response is a crucial treatment for fungal keratitis. NPD1 is a lipid mediator that promotes inflammation-resolution. Previous studies have proved that NPD1 can play a strong anti-inflammatory role in different nervous system diseases and retinal diseases[30-31]. Our data showed that NPD1 treatment reduced the degree of corneal infiltration and ulcer in *A. fumigatus* infected mice corneas and reduced corneal tissue damage. This is consistent with another study, which demonstrated that the local application of methyl ester prodrug NPD1 reduced the incidence rate and degree of corneal damage as well as reduced the degree of corneal neovascularization in herpes simplex virus-induced stromal keratitis[32]. These results suggest that NPD1 can promote the resolution of inflammation response in infectious keratitis.

Innate immunity is the first line of defense against fungal infection. When the pattern recognition receptor recognizes the invaded fungus, the innate immune response is immediately initiated and resulted in the release of pro-inflammatory factors such as IL-1β, IL-6, IL-8, and IFN-γ[7,33-34]. IL-1β is considered a marker of inflammation and reducing the production of IL-1β significantly reduced corneal opacity in fungal keratitis[8-9,35]. Our research data showed that NPD1

effectively curbed the expression of pro-inflammatory factors IL-1β and MIP-2 in *A. fumigatus* infected corneas. Our data is consistent with a published paper which argued that NPD1 can reduce the expression of TNF-α and IL-1β, and thus protect photoreceptors away from apoptosis[36]. More interestingly, we also observed that NPD1 promoted the expression of the anti-inflammatory signaling pathway Nrf-2/HO-1 and inhibit the activation of the pro-inflammatory signaling pathway p38 MAPK. Studies have proved that both inhibition of the p38 MAPK pathway and activation of the Nrf-2/HO-1 pathway can significantly slow down the course of *A. fumigatus* keratitis and improve prognosis[26,28,37]. The evidence above suggests that NPD1 can inhibit *A. fumigatus* induced corneal inflammatory response by regulating the balance of pro- and anti-inflammatory signal pathways.

Host anti-fungal immune responses in the cornea are mainly undertaken by innate immunity mediated by macrophages and neutrophils. Neutrophils are the first immune cells to be recruited into the infected cornea. By releasing cytotoxic molecules, respiratory burst, and neutrophil extracellular trap, neutrophils can effectively eliminate the invaded pathogenic bacteria, but also damage the corneal tissue structure[4,38-39]. Our study shows that NPD1 can effectively inhibit the recruitment of neutrophils and reduced the activity of the MPO enzyme after corneas are infected with *A. fumigatus*. This coincides with another study which demonstrated that NPD1 has anti-inflammatory activity, and can decrease neutrophil infiltration and T cell migration in the nervous systems and immune systems[21]. Macrophages, as the first-line defense cells, can polarize into different phenotypes in response to local microenvironment signals, including M1 (pro-inflammatory) and M2 macrophages (anti-inflammatory)[40-42]. M1 macrophages facilitated the clearance of pathogenic fungi in the early stages of infection, however, it will lead to uncontrolled inflammation and tissue damage if the M1 macrophages polarization keeps for a long time[43]. M2 macrophages are associated with anti-inflammatory response and tissue remodeling[44]. Studies have reported that M2 macrophages play a protective role in keratitis[45]. Our results show that NPD1 can reduce the total number of macrophages and promote its transformation to M2 type, and thus raised the ratio of M1/M2 macrophages in *A. fumigatus* infected cornea. A previous study has shown that NPD1 treatment could increase the expression level of CD206 (M2 marker) and regulate the expression of inflammatory cytokines in lipopolysaccharide (LPS)-stimulated macrophages[46], which is consistent with us. This polarization shift is likely mechanistically linked to our other findings. The observed inhibition of the pro-inflammatory p38 MAPK pathway, which is a known driver of M1 polarization, coupled with the activation of the Nrf-2/HO-1 anti-inflammatory axis, likely creates a local microenvironment that actively suppresses M1 characteristics while promoting the M2 phenotype. Thus, NPD1 does not just passively reduce inflammation; it actively orchestrates the resolution phase by reprogramming key immune cells. The above results prove that NPD1 can play an anti-inflammatory role by suppressing the aggregation of neutrophils and macrophages, and increasing the polarization of macrophages to M2 type in fungal keratitis.

In addition, cornea is densely innervated with sensory nerves. The homeostasis of the ocular surface and the repair of corneal injury partly depends on the relationship between corneal epithelial cells and corneal nerves. As we all know, corneal nerves can promote epithelial cell appreciation and differentiation, as well as maintain the mechanical sensitivity thresholds of the cornea[10,47]. However, it has been proved that the degree of corneal innervation was reduced in *A. fumigatus* keratitis[6]. In our experiment, we found that NPD1 can effectively promote the repair of damaged nerves and restore corneal sensitivity in *A. fumigatus* keratitis. A previous study has argued that the simultaneous application of pigment epithelium-derived factor (PEDF) and DHA could increase the synthesis of NPD1 after corneal stromal dissection, followed by the regeneration of corneal nerve, while neither PEDF nor DHA alone has the same effects[48]. This is consistent with us and proves that NPD1 can promote the restoration of damaged corneal nerves in *A. fumigatus* keratitis. Furthermore, we found that NPD1 treatment significantly improved the expression of CGRP. As an important corneal neuropeptide, CGRP plays an important role in the corneal neurotrophic function and immune regulation[49-50]. The elevation of CGRP is a key piece of the puzzle. CGRP is not only a marker for sensory nerves but is also a potent neurotrophic and immunomodulatory factor itself. By promoting CGRP expression, NPD1 may indirectly create a positive feedback loop: NPD1 promotes nerve survival, which in turn leads to more CGRP release, and this CGRP further supports nerve regeneration and helps to dampen the local inflammatory response, creating a more permissive environment for tissue healing. This proved that NPD1 indirectly protects the cornea by inducing corneal nerve regeneration in *A. fumigatus* keratitis.

Our study, for the first time, unveils the unique therapeutic potential of NPD1 in these complex pathological processes, thereby highlighting the innovativeness and significance of this work.

In summary, we found that NPD1 can repress the inflammatory response of *A. fumigatus* keratitis, and promote tissue repair and corneal nerve regeneration. Our data showed that NPD1 can effectively reduce the expression of IL-1β and MIP-2, inhibit the activation of the pro-inflammatory p38 MAPK signaling pathway, and increase the expression of the anti-

inflammatory Nrf-2/HO-1 signaling pathway in *A. fumigatus* infected corneas. In addition, NPD1 can reduce the recruitment of neutrophils and macrophages as well as promote the M2 macrophages polarization. Therefore, NPD1 is expected to be a new option for regulating the excessive inflammatory response of *A. fumigatus* keratitis.

ACKNOWLEDGEMENTS

Authors' Contributions: Zhao GQ and Li C conceived of the study and participated in its design and coordination. Lin J and Zhang LN participated in the design of the research. Jia YY performed the statistical analysis. Li H carried out the experiments and drafted the manuscript. Wu MQ helped preparing *A. fumigatus* antigen. Wang Q helped drafting the manuscript. All authors read and approved the final manuscript.

Foundations: Supported by National Natural Science Foundation of China (No.81870632; No.81800800; No.82171029); Taishan Scholars Program of Shandong Province, China (No.tsqn202103188).

Conflicts of Interest: Li H, None; **Jia YY,** None; **Wu MQ,** None; **Zhang LN,** None; **Lin J,** None; **Wang Q,** None; **Li C,** None; **Zhao GQ,** None.

REFERENCES

1 Awad R, Ghaith A, Awad K, *et al*. Fungal keratitis: diagnosis, management, and recent advances. *Clin Ophthalmol* 2024;18:85-106.

2 Shareef O, Shareef S, Saeed HN. New frontiers in acanthamoeba keratitis diagnosis and management. *Biology* 2023;12(12):1489.

3 Burn H, FlorCruz NV, Hoffman J. Medical interventions for fungal keratitis. *Cochrane Database Syst Rev* 2025;2025(10):CD016304.

4 Zhao WY, Che CY, Liu KX, *et al*. Fenretinide inhibits neutrophil recruitment and IL-1β production in aspergillus fumigatus keratitis. *Cornea* 2018;37(12):1579-1585.

5 Ling JYM, Yeung SN, Chan CC, *et al*. Trends and clinical outcomes of fungal keratitis in Canada: a 20-year retrospective multicentre study. *Am J Ophthalmol* 2024;265:147-155.

6 Ren CJ. Changes in corneal innervation and pain responses in fungal keratitis. *Int J Ophthalmol* 2020;13(1):1-6.

7 Sharma N, Bagga B, Singhal D, *et al*. Fungal keratitis: a review of clinical presentations, treatment strategies and outcomes. *Ocul Surf* 2022;24:22-30.

8 Sun Y, Abbondante S, Karmakar M, *et al*. Neutrophil caspase-11 is required for cleavage of caspase-1 and secretion of IL-1β in *Aspergillus fumigatus* infection. *J Immunol* 2018;201(9):2767-2775.

9 Mpakosi A, Kaliouli-Antonopoulou C. Immune mechanisms of filamentous fungal keratitis. *Cureus* 2024:e61954.

10 Ueno H, Ferrari G, Hattori T, *et al*. Dependence of corneal stem/progenitor cells on ocular surface innervation. *Invest Ophthalmol Vis Sci* 2012;53(2):867-872.

11 Asiedu K. The role of corneal nerves and neuromodulators in the immune response and healing processes of corneal infections. *Exp Eye Res* 2025;258:110498.

12 Zia B, Elmeky M, Azimullah S, *et al*. The multifaceted role of neuroprotectin D1: physiological, pathophysiological, and pharmacological insights in neurodegenerative diseases. *Curr Neuropharmacol* 2025;23(10):1215-1231.

13 Hong S, Gronert K, Devchand PR, *et al*. Novel docosatrienes and 17S-resolvins generated from docosahexaenoic acid in murine brain, human blood, and glial cells. *J Biol Chem* 2003;278(17):14677-14687.

14 Marcheselli VL, Hong S, Lukiw WJ, *et al*. Novel docosanoids inhibit brain ischemia-reperfusion-mediated leukocyte infiltration and pro-inflammatory gene expression. *J Biol Chem* 2003;278(44):43807-43817.

15 Serhan CN. Resolution phase of inflammation: novel endogenous anti-inflammatory and proresolving lipid mediators and pathways. *Annu Rev Immunol* 2007;25:101-137.

16 Kanan Y, Gordon WC, Mukherjee PK, *et al*. Neuroprotectin D1 is synthesized in the cone photoreceptor cell line 661W and elicits protection against light-induced stress. *Cell Mol Neurobiol* 2015;35(2):197-204.

17 Bazan NG, Molina MF, Gordon WC. Docosahexaenoic acid signalolipidomics in nutrition: significance in aging, neuroinflammation, macular degeneration, Alzheimer's, and other neurodegenerative diseases. *Annu Rev Nutr* 2011;31:321-351.

18 Qin Q, Patil KA, Gronert K, *et al*. Neuroprotectin D1 inhibits retinal ganglion cell death following axotomy. *Prostaglandins Leukot Essent Fat Acids* 2008;79(6):201-207.

19 Mukherjee PK, Marcheselli VL, Serhan CN, *et al*. Neuroprotectin D1: a docosahexaenoic acid-derived docosatriene protects human retinal pigment epithelial cells from oxidative stress. *Proc Natl Acad Sci U S A* 2004;101(22):8491-8496.

20 Schwab JM, Nan CA, Arita M, *et al*. Resolvin E1 and protectin D1 activate inflammation-resolution programmes. *Nature* 2007;447(7146):869-874.

21 Hamidzadeh K, Westcott J, Wourms N, *et al*. A newly synthesized 17-epi-NeuroProtectin D1/17-epi-Protectin D1: Authentication and functional regulation of Inflammation-Resolution. *Biochem Pharmacol* 2022;203:115181.

22 González-Périz A, Planagumà A, Gronert K, *et al*. Docosahexaenoic acid (DHA) blunts liver injury by conversion to protective lipid mediators: protectin D1 and 17S-hydroxy-DHA. *FASEB J* 2006;20(14):2537-2539.

23 Kenchegowda S, He J, Bazan HEP. Involvement of pigment epithelium-derived factor, docosahexaenoic acid and neuroprotectin D1 in corneal inflammation and nerve integrity after refractive surgery. *Prostaglandins Leukot Essent Fat Acids* 2013;88(1):27-31.

24 Cortina MS, He JC, Russ T, *et al*. Neuroprotectin D1 restores corneal nerve integrity and function after damage from experimental surgery. *Invest Ophthalmol Vis Sci* 2013;54(6):4109-4116.

25 Abbondante S, Leal SM, Clark HL, *et al*. Immunity to pathogenic fungi in the eye. *Semin Immunol* 2023;67:101753.

26 Jia YY, Li C, Yin M, *et al*. Kaempferol ameliorate the prognosis of *Aspergillus fumigatus* keratitis by reducing fungal load and inhibiting the Dectin-1 and p38 MAPK pathway. *Exp Eye Res* 2022;216:108960.

27 Jiang N, Zhang L, Zhao GQ, *et al*. Indoleamine 2, 3-dioxygenase regulates macrophage recruitment, polarization and phagocytosis in *Aspergillus fumigatus* keratitis. *Invest Ophthalmol Vis Sci* 2020;61(8):28.

28 Gu LW, Lin J, Wang Q, *et al*. Dimethyl itaconate protects against fungal keratitis by activating the Nrf2/HO-1 signaling pathway. *Immunol Cell Biol* 2020;98(3):229-241.

29 Zhou YF, Lin J, Peng XD, *et al*. The role of netrin-1 in the mouse cornea during *Aspergillus fumigatus* infection. *Int Immunopharmacol* 2019;71:372-381.

30 Asatryan A, Calandria JM, Kautzmann MI, *et al*. New retinal pigment epithelial cell model to unravel neuroprotection sensors of neurodegeneration in retinal disease. *Front Neurosci* 2022;16:926629.

31 Calandria JM, Do KV, Kala-Bhattacharjee S, *et al*. cRel and Wnt5a/frizzled 5 receptor-mediated inflammatory regulation reveal novel neuroprotectin D1 targets for neuroprotection. *Cell Mol Neurobiol* 2023;43(3):1077-1096.

32 Rajasagi NK, Reddy PBJ, Mulik S, *et al*. Neuroprotectin D1 reduces the severity of herpes simplex virus–induced corneal immunopathology. *Invest Ophthalmol Vis Sci* 2013;54(9):6269-6279.

33 Roilides E, Antachopoulos C, Simitsopoulou M. Pathogenesis and host defence against Mucorales: the role of cytokines and interaction with antifungal drugs. *Mycoses* 2014;57(s3):40-47.

34 Zhang YN, Liang QF, Liu Y, *et al*. Expression of cytokines in aqueous humor from fungal keratitis patients. *BMC Ophthalmol* 2018;18(1):105.

35 Hardison SE, Brown GD. C-type lectin receptors orchestrate antifungal immunity. *Nat Immunol* 2012;13(9):817-822.

36 Sheets KG, Zhou Y, Ertel MK, *et al*. Neuroprotectin D1 attenuates laser-induced choroidal neovascularization in mouse. *Mol Vis* 2010;16:320-329.

37 Yu B, Li C, Gu LW, *et al*. Eugenol protects against *Aspergillus fumigatus* keratitis by inhibiting inflammatory response and reducing fungal load. *Eur J Pharmacol* 2022;924:174955.

38 Zhao GQ, Hu M, Li C, *et al*. Osteopontin contributes to effective neutrophil recruitment, IL-1β production and apoptosis in *Aspergillus fumigatus* keratitis. *Immunol Cell Biol* 2018;96(4):401-412.

39 Niu YW, Zhao GQ, Li C, *et al*. *Aspergillus fumigatus* increased PAR-2 expression and elevated proinflammatory cytokines expression through the pathway of PAR-2/ERK1/2 in cornea. *Invest Ophthalmol Vis Sci* 2018;59(1):166-175.

40 Ríos JD, Hughes CK, Lally J, *et al*. Neuroprotectin D1 attenuates blast overpressure induced reactive microglial cells in the cochlea. *Laryngoscope* 2021;131(6): E2018-E2025.

41 Lendeckel U, Venz S, Wolke C. Macrophages: shapes and functions. *ChemTexts* 2022;8(2):12.

42 Cerdeira CD, Brigagão MRPL. Targeting macrophage polarization in infectious diseases: M1/M2 functional profiles, immune signaling and microbial virulence factors. *Immunol Investig* 2024;53(7):1030-1091.

43 Liu GB, Dong CX, Chen B, *et al*. Dectin-1 participates in the immune-inflammatory response to mouse *Aspergillus fumigatus* keratitis by modulating macrophage polarization. *Front Immunol* 2024;15:1431633.

44 Xue JD, Gao J, Tang AF, *et al*. Shaping the immune landscape: Multidimensional environmental stimuli refine macrophage polarization and foster revolutionary approaches in tissue regeneration. *Heliyon* 2024;10(17):e37192.

45 Wang ZY, Lin J, Wang Q, *et al*. Rosmarinic acid alleviates fungal keratitis caused by *Aspergillus fumigatus* by inducing macrophage autophagy. *Exp Eye Res* 2024;244:109944.

46 Zhou Y, Wang J, Li X, *et al*. Neuroprotectin D1 protects against postoperative delirium-like behavior in aged mice. *Front Aging Neurosci* 2020;12:582674.

47 Shadmani A, Wu AY. Navigating the path to corneal healing success and challenges: a comprehensive overview. *Eye (Lond)* 2025;39(6):1047-1055.

48 Cortina MS, He JC, Li N, *et al*. Neuroprotectin D1 synthesis and corneal nerve regeneration after experimental surgery and treatment with PEDF plus DHA. *Invest Ophthalmol Vis Sci* 2010;51(2):804-810.

49 Yin M, Li C, Peng XD, *et al*. Expression and role of calcitonin gene-related peptide in mouse *Aspergillus fumigatus* keratitis. *Int J Ophthalmol* 2019;12(5):697-704.

50 Zidan AA, Zhu SY, Elbasiony E, *et al*. Topical application of calcitonin gene-related peptide as a regenerative, antifibrotic, and immunomodulatory therapy for corneal injury. *Commun Biol* 2024;7:264.