International Immunopharmacology 69 (2019) 103–108

Contents lists available at ScienceDirect

International Immunopharmacology

journal homepage: www.elsevier.com/locate/intimp

Maresin1 regulates neutrophil recruitment and IL-10 expression in *Aspergillus fumigatus* keratitis

Qing Tang[a], Chengye Che[a], Jing Lin[a], Hong He[b], Wenyi Zhao[a], Leyu Lv[a], Guiqiu Zhao[a,*]

[a] Department of Ophthalmology, The Affiliated Hospital of Qingdao University, Qingdao, Shandong Province, China
[b] Clinical Laboratory, The Affiliated Hospital of Qingdao University, Qingdao, Shandong Province, China

ARTICLE INFO

Keywords:
Fungal keratitis
Aspergillus fumigatus
Maresin1
Cytokines
Inflammatory cells

ABSTRACT

Purpose: Maresin1, a lipid mediator derived from polyunsaturated fatty acids, has been shown to suppress the inflammatory response in various inflammatory diseases. However, its effects in fungal keratitis are still uncertain. In this study, we investigated the role of maresin1 (MaR1) in *Aspergillus fumigatus* keratitis of the eye in a mouse model.

Methods: Mouse corneas were infected with *A. fumigatus* by corneal intrastromal injection. Two hours after infection, maresin1 (5 ng/5 μl) was delivered by subconjunctival injection. Then, topical administration of maresin1 (5 ng/3 μl) was applied to mouse corneas twice a day from day 1 to day 5. The development of FK lesions, the production of chemokines, the production of inflammation cytokines and the levels of p-GSK3β were measured via slit-lamp biomicroscope, quantitative polymerase chain reaction (qRT-PCR) and western blot. The presence of neutrophils in the cornea was detected by immunofluorescence staining and myeloperoxidase. The effect of maresin1 on *A. fumigatus* stimulated mouse macrophage RAW264.7 cells was assessed via PCR and Western blot.

Results: In our study, administration of maresin1 reduced the severity of fungal keratitis with infiltration of fewer neutrophils and reduced levels of the chemokine CXCL1, while the anti-inflammatory cytokines such as IL-10 were enhanced compared with the PBS group. Additionally, in vitro studies showed that treatment with maresin1 inhibited the production of the chemokine CXCL1 and enhanced IL-10 levels in *A. fumigatus* stimulated RAW264.7 mouse macrophages. Moreover, levels of p-GSK3β increased after maresin1 treatment in *A. fumigatus* stimulated RAW264.7 cells.

Conclusion: Taken together, these findings demonstrate that treatment with maresin1 moderates corneal inflammation through reducing neutrophil recruitment and levels of the chemokine CXCL1 and enhancing the anti-inflammatory cytokine IL-10 in *A. fumigatus* keratitis. Additionally, maresin1 alters levels of GSK3β phosphorylation to regulate CXCL1 and IL-10 expression in response to *A. fumigatus* infection. Topical administration of maresin1 may emerge as a novel anti-inflammatory molecule and has a protective role in *A. fumigatus* keratitis.

1. Introduction

Fungal keratitis is a chronic intractable lesion of the eye caused by pathogenic fungi, and it has high morbidity in developing countries [1]. With the increasing use of antibiotic drugs and excessive corticosteroids, fungal keratitis has become a common cause of blindness in many countries [2]. Management of corneal inflammation always relies on anti-fungal drugs such as Natamycin and Voriconazole [3]. However, treatment is still troublesome because antifungal agents always have side effects and are not effective in fungal keratitis, which limits their application [4].

Both pathogenic fungi and immune damage are involved in corneal injury. Innate immunity is the first line of host defense to control infections [5]. Pattern recognition receptors identify specific structures in microbial pathogens and then produce chemokines and cytokines to recruit inflammatory cells to eliminate pathogens, which is important to maintain the normal structure of the cornea [6]. However, an excessive immune response also causes an inflammatory disorder that is harmful to the host. Therefore, there is an urgent need to exploit a novel approach to modulate excessive inflammation but not influence the elimination of pathogens.

Interestingly, the host can endogenously produce numerous

* Corresponding author.
E-mail address: zhaoguiqiu_good@126.com (G. Zhao).

https://doi.org/10.1016/j.intimp.2019.01.032
Received 8 October 2018; Received in revised form 19 December 2018; Accepted 22 January 2019
Available online 25 January 2019
1567-5769/

molecules to regulate the inflammatory response and accelerate resolution [7]. These products are found in inflammatory exudate when inflammation begins to resolve and tissues start to repair themselves [8]. Maresin1 is one such lipid molecule which is synthesized from an omega-3 polyunsaturated fatty acid, docosahexaenoic acid (DHA), in macrophages [9]. Several studies have confirmed that maresin1 has the ability to control inflammatory disorders and possesses pro-resolution properties that can eventually lead to tissue homeostasis. The effects of maresin1 were largely linked with its ability to inhibit neutrophil recruitment, reduce production of several proinflammatory cytokines and chemokines, improve the Treg/TH17 imbalance, increase phagocytosis of apoptotic neutrophils and zymosan and attenuate endoplasmic reticulum stress [10–15]. Since many of these activities are involved in fungal corneal injury, we tested the effect of maresin1 in chronic inflammation caused by *A. fumigatus* infection.

In this report, we demonstrate that administration with maresin1 was effective in mouse *A. fumigatus* keratitis. Administration with maresin1 decreased the disease severity in mice corneas at 3 d post-infection. The results also showed that maresin1 dampened the influx of neutrophils into the corneal stroma. Additionally, production of the chemokine CXCL1 was decreased while production of the anti-inflammatory cytokine IL-10 was enhanced. GSK3β was also phosphorylated and lost its original function of promoting an inflammatory reaction with maresin1 treatment. These data show that administration of maresin1 plays a protective role in *A. fumigatus* keratitis.

2. Materials and methods

2.1. Murine models of corneal inflammation

Specific pathogen-free (SPF) C57BL/6 mice (female, 8 weeks) were purchased from Jinan Pengyue Laboratory Animal Ltd. (Jinan, China). The standard *A. fumigatus* strain was purchased by the China General Microbiological Culture Collection Center (Beijing, China), strain 3.0772. 8% chloral hydrate (0.08 ml/mouse) was used to anesthetize mice by intraperitoneal injection. An abrasion was made by a 30-gauge needle to the depth of the superficial stroma. Then, through the experimental hole, 2.5 μl of conidial suspension (2.5×10^5 conidia/μl PBS) were injected into the stroma of corneas with a 33-gauge Hamilton syringe. All treatment of mice complied with the regulations of the Chinese Ministry of Science and Technology Guidelines on the Humane Treatment of Laboratory Animals (vGKFCZ-2006–398) and were in line with the Statement on the Use of Animals in Ophthalmic and Vision Research declared by the Association for Research in Vision and Ophthalmology (ARVO).

2.2. Maresin1 administration

Two hours after infection, 5 μl of 5 ng maresin1 (Cayman) was given to the left eyes ($n = 6$/group) via subconjunctival injection. Maresin1 was topically applied to the cornea (5 ng per eye; 3 μl drop) twice a day from day 1 to day 5 until the infection resolved. The vehicle control group received an equal volume of phosphate-buffered saline (PBS) from day 1 to day 5.

2.3. Cell culture and A. fumigatus stimulation

RAW264.7 (obtained from Shanghai Chinese Academy of Sciences) murine macrophage cells were cultured in High Glucose Dulbecco's Minimal Essential Medium (Gibco, USA) supplemented with 12% fetal bovine serum (FBS), and incubated at 37 °C in 5% CO_2. Near 80% confluence, the cells were pretreatment with or without 10 nM maresin1 for 2 h, and then incubated with *A. fumigatus* hyphae (to the final concentration of 5×10^6 CFU/mL) in twelve-well plates and six-well plates for 4 h and 20 h for qPCR and Western blot, respectively.

Table 1
Nucleotide sequences of mouse primers for real-time RT-PCR.

Gene	GenBank no.	Primer sequence (5′–3′)
mβ-Actin	NM_007393.5	F: GATTACTGCTCTGGCTCCTAGC R: GACTCATCGTACTCCTGCTTGC
mIL-10	NM_010548.2	F: TGCTAACCGACTCCTTAATGCAGGAC R: CCTTGATTTCTGGGCCATGCTTCTC
mCXCL1	NM_008176.3	F: TCACCTCAAGAACATCCAGAGC R: ACTTGGGGACACCTTTTAGCAT

2.4. Quantitative polymerase chain reaction (qRT-PCR)

Total RNA was extracted from mouse corneas and RAW264.7 cells using the RNAiso Plus reagent (Takara) and was rapidly quantified by spectrophotometry (Eppendorf). cDNA was obtained by reverse transcription of 2 μg of total RNA using the Primescript RT Reagent Kit (Takara). Quantitative RT-PCR was performed using an Eppendorf Mastercycler and SYBR green. β-Actin was used as an internal control. The sequences of oligonucleotide primers are shown in Table 1.

2.5. Western blotting

Corneas and cells were homogenized in radioimmunoprecipitation assay (RIPA; Solarbio) lysis buffer containing phosphatase inhibitor cocktail (MCE) and phenylmethanesulfonyl fluoride (PMSF; Solarbio) (100:1:1). Total protein was separated on SDS-PAGE gels (12%) and transferred to PVDF membranes (Millipore). Then the membrane was incubated with blocking buffer (Beyotime) for 2 h. The membrane was incubated overnight with the primary antibody of the target protein. Washing with PBS containing 0.05% Tween-20 for three times, membrane was incubated with second antibody. Primary antibodies against the following proteins were used: IL-10 (Cell Signaling Technology), CXCL-1 (Proteintech), GSK3β (Elbscience) and p-GSK3β (Elbscience). Secondary antibodies included HRP-linked anti-rabbit (Elbscience) and anti-mouse (Elbscience) antibodies. HRP conjugated anti-β-actin (Bioss) was set for a control. The immunoreactive bands were visualized with ECL reagents (Beyotime).

2.6. Immunofluorescence staining

The protein expression and localization of NIMP-R14 in mouse corneas was observed by immunofluorescence staining. Eyeballs were removed at 3 d post-infection from C67BL/6 mice, embedded in optimum cutting temperature (O.C.T.) compound (Sakura Finetek USA, Inc.) and then rapidly frozen in liquid nitrogen. Then, 10-μm slices were fixed in acetone for 5 min. The slices were blocked with 10% donkey serum (Solarbio) for 30 min at room temperature. To label PMNs, the slices were incubated at a 1:300 dilution of rat anti-mouse NIMP-R14 antibody (Santa Cruz Biotechnology) at 37 °C for 3 h. After being washed with PBS, slices were stained with goat anti-rat IgG (1:300, Cwbio, Wuhan, China) for 1 h. Finally, slices were visualized and digital images were captured with a Zeiss Axiovert microscope at ×200 magnification.

2.7. Quantitation of corneal neutrophils

Neutrophil infiltration into the corneal stroma was assessed indirectly by measuring myeloperoxidase (MPO) activity. Corneas ($n = 6$/group/time) were removed at 3 d post-infection, and homogenized in 1.0 ml of the second agent of the MPO test kit (Njjcbio, Nanjing, China) according to the manufacturer's instructions. Samples were freeze-thawed and centrifuged, and then supernatant were warmed in a water bath, the change in absorbency (460 nm) was immediately monitored. The slope of the line was determined for each sample and used to calculate units of MPO per cornea.

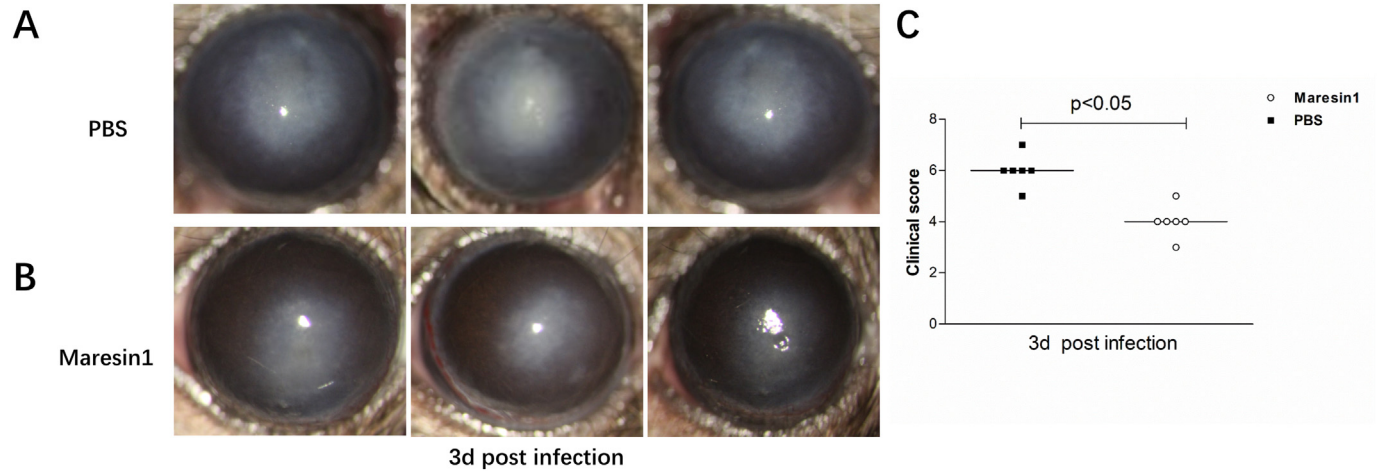

Fig. 1. (A–B) Treatment with maresin1 reduces the inflammatory response in mouse *A. fumigatus* keratitis. Compared with PBS group (B), maresin1 (A) significantly reduced disease severity in the cornea from photographs taken by slit lamp at 3 days post-infection. Maresin1 also decreased clinical score compared with PBS group (C).

2.8. GSK3β inhibitor treatment of RAW264.7 cells

RAW264.7 cells were pretreated with GSK3β inhibitor LiCL (solarbio; 20 nM) 2 h previous to the stimulation of *A. fumigatus*. Then 10 nM maresin1 was pretreated with RAW264.7 cells 1 h before *A. fumigatus* stimulation. Control group were cultured with PBS.

2.9. Statistical analysis

An unpaired, two-tailed Student's *t*-test was used to determine the statistical significance of RT-PCR and Western blot data. These data were represented as the mean ± SD and were analyzed by GraphPad 7.0 software. Differences were considered significant at $p \leq 0.05$.

3. Results

3.1. Disease response after maresin1 treatment

The role of maresin1 in reducing an inflammatory response was demonstrated by photographs taken by silt lamp (Fig. 1A–B) and by clinical scores (Fig. 1C; $p < 0.05$). The results showed that treatment with maresin1 significantly reduced the disease severity of mouse *A. fumigatus* keratitis when compared with the PBS group at 3 d post-infection. An obvious difference was also observed in clinical scores of the maresin1 treated group versus the PBS treated group ($p < 0.05$).

3.2. Maresin1 inhibits neutrophil recruitment to the corneal stroma in mouse A. fumigatus keratitis

Immunostaining was performed to detect changes in neutrophils in *A. fumigatus* infected corneas after maresin1 treatment at 3 d post-infection. Immunostaining demonstrated that after maresin1 treated (Fig. 2A), PMN (red) numbers were significantly decreased when compared with the PBS group (Fig. 2B) at 3 d post-infection. Treatment with maresin1 significantly reduced MPO levels (Fig. 2C; $p < 0.001$) compared with the PBS group.

3.3. Maresin1 regulates CXCL1 and IL-10 expression in mouse A. fumigatus keratitis

Maresin1 treatment significantly enhanced IL-10 mRNA levels (Fig. 3A; $p < 0.001$) induced by *A. fumigatus* at 1 d, 3 d and 5 d post-infection. Moreover, protein levels of IL-10 (Fig. 3B; $p < 0.001$) were elevated after maresin1 treatment at 1 d, 3 d and 5 d post-infection. In contrast to IL-10, mRNA levels of the chemokine CXCL1 (Fig. 3C; $p < 0.001$) induced by *A. fumigatus* were reduced by maresin1 treatment at 3 d and 5 d post-infection. Additionally, protein levels of CXCL1 (Fig. 3D; $p < 0.001$) were also inhibited by maresin1 at 3 d and 5 d post-infection when compared with the PBS group.

3.4. Maresin1 regulates CXCL1 and IL-10 expression in RAW264.7 cells stimulated by A. fumigatus

Treatment with maresin1 significantly reduced mRNA levels of CXCL1 (Fig. 4A; $p < 0.001$) induced by *A. fumigatus* compared with PBS treatment. Additionally, protein levels of CXCL1 (Fig. 4B; $p < 0.001$) were also reduced by treatment of maresin1 (Fig. 4C; $p < 0.001$). Another result showed that mRNA levels of IL-10 were enhanced after maresin1 intervention. Treatment with maresin1 also enhanced IL-10 protein levels (Fig. 4C; $p < 0.001$).

3.5. Maresin1 regulates CXCL1 and IL-10 by inhibiting GSK3β activity in RAW264.7 cells stimulated by A. fumigatus

We further investigated how maresin1 regulates inflammatory cytokine production. We assayed p-GSK3β levels in *A. fumigatus* stimulated RAW264.7 cells with and without maresin1 pretreatment via Western blot. The results showed that maresin1 increased p-GSK3β protein levels in *A. fumigatus* stimulated RAW264.7 cells at 60 min post-infection (Fig. 5A; $p < 0.01$). Next, we sought to determine whether the expression of CXCL1 and IL-10 were regulated by GSK3β with maresin1 pretreatment. We demonstrated that the GSK3β inhibitor, LiCl, reduced mRNA levels of CXCL1 (Fig. 5B; $p < 0.001$) and enhanced levels of IL-10 (Fig. 5C; $p < 0.001$). Additionally, the suppression of CXCL1 levels were further enhanced (Fig. 5B; $p < 0.001$) by maresin1 in *A. fumigatus* stimulated RAW264.7 cells. What's more, the secretion of anti-inflammatory cytokine IL-10 was further upregulated when combined with maresin1 and LiCl pretreatment (Fig. 5C; $p < 0.001$).

4. Discussion

Fungal infection of the eye results in a chronic inflammatory reaction in the cornea that always causes ulcers and perforation [16]. In this report, we investigated the function of maresin1 in controlling fungal keratitis lesions using a mouse model of *A. fumigatus* keratitis. Previous studies have demonstrated that additional DHA lipid-derived mediators, such as resolve D1 and aspirin-triggered resolve D1, as well as

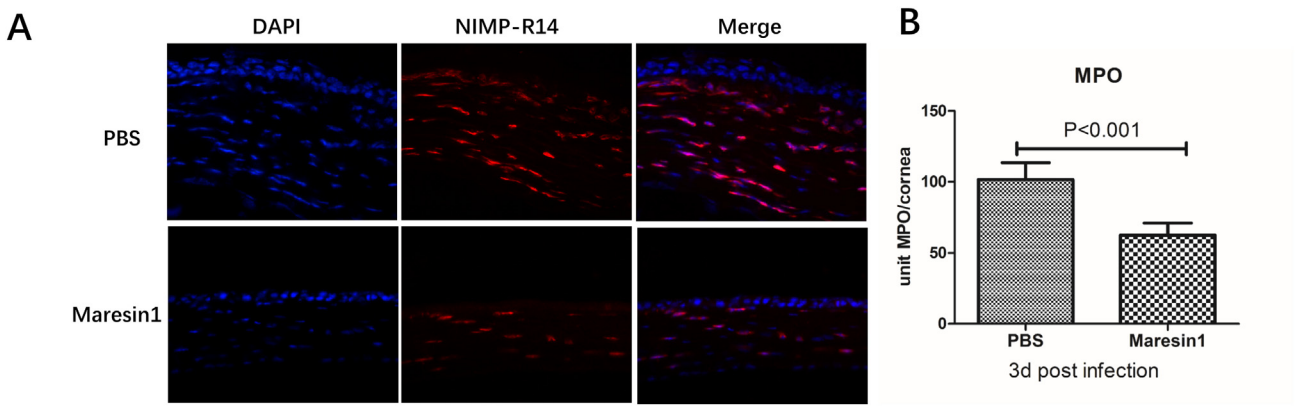

Fig. 2. (A–B) Treatment with maresin1 reduces neutrophil influx into the corneal stroma. Images were taken by a slit lamp at 3 d post-infection (A). Neutrophils were labeled with the NIMP-R14 marker, and the numbers of neutrophils decreased compared with the PBS group after maresin1 treatment. The number of neutrophils with or without maresin1 treatment in the cornea was counted by myeloperoxidase (B).

mediators such as EPA-derived RvE1, have a protective role in corneal inflammation [17]. In this study, we found that a new DHA-derived molecule maresin1 can act as a modulator of inflammation in corneal inflammation. Rodrigo et al. [18] found that treatment with maresin1 could protect mice against DSS-induced colitis with small changes in body weight and colon length compared with the control group, and Li [19] proved that maresin1 could improve survival rates and mitigate inflammation in a sepsis model. Our results showed that the extent of corneal opacity and other FK lesions were reduced by administration of maresin1 at 3 days post-infection.

Additionally, administration of maresin1 dampens inflammatory action by inhibiting several key events involved in FK pathogenesis. Neutrophil infiltration and activation in the corneal stroma are important pathophysiological features of *A. fumigatus* keratitis [20]. Although the participation of neutrophils in acute inflammation is often beneficial, it can also contribute to processes that impair normal tissues under special circumstances [21,22]. Their paradoxical role in inflammation indicates that excessive neutrophil infiltration is bad for tissue homeostasis [23]. In our study, we found that treatment with maresin1 inhibited the influx of neutrophils into the corneal stroma during *A. fumigatus* infection. Rodrigo et al. [12] believed that treatment of maresin1 reduced the migration of neutrophils and protected mice from immune damage during colitis.

Chemokines are chemotactic cytokines that control the migration and trafficking behavior of peripheral immune cells [24]. After chemokine and cytokine stimulation, neutrophils are recruited into the corneal stroma and play a vital role during infection with *A. fumigatus* [25]. As a member of the chemokine family, CXCL1 plays a vital role in

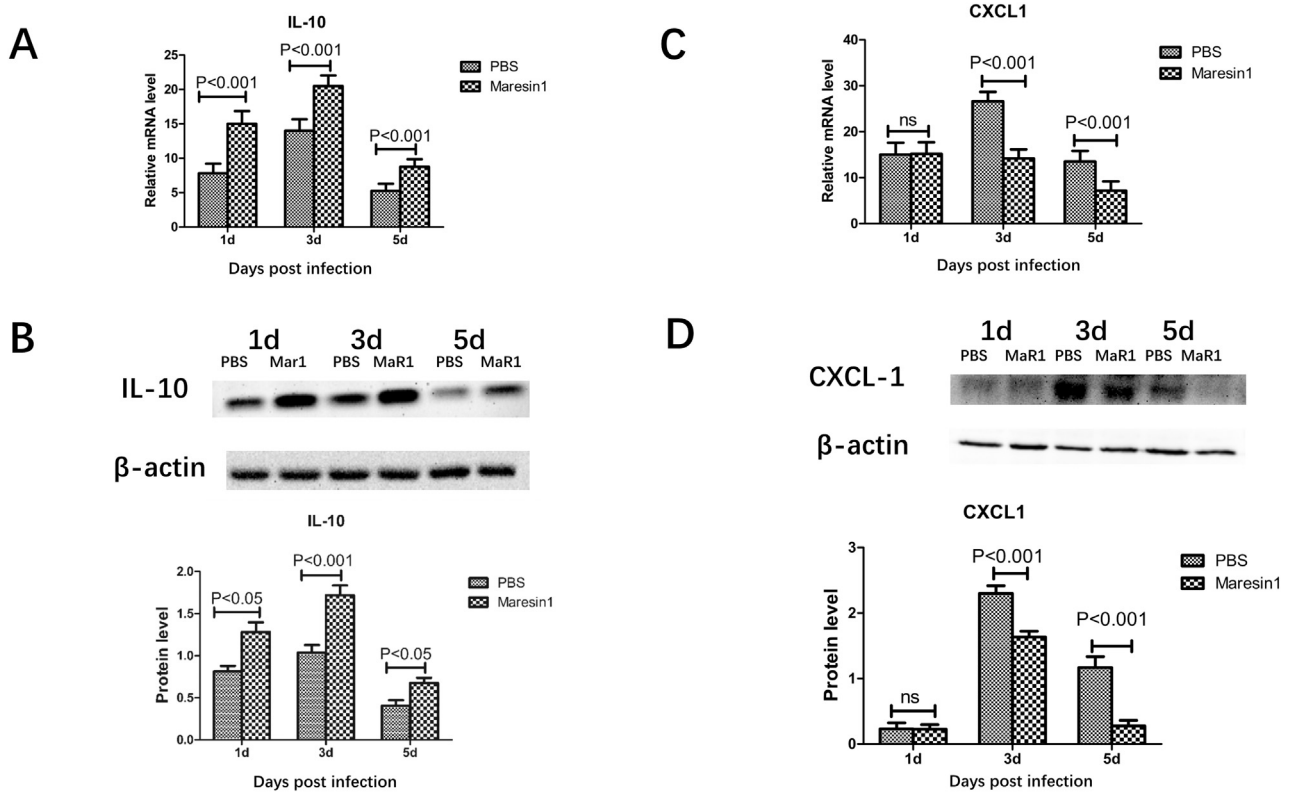

Fig. 3. (A–D) Maresin1 regulates IL-10 and CXCL1 expression in *A. fumigatus* keratitis. Treatment of maresin1 upregulated mRNA levels (A) and protein levels (B) of IL-10 at 1 d, 3 d and 5 d post-infection. Moreover, mRNA levels (C) and protein levels (D) of CXCL1 were reduced at 3 d and 5 d post-infection with *A. fumigatus*.

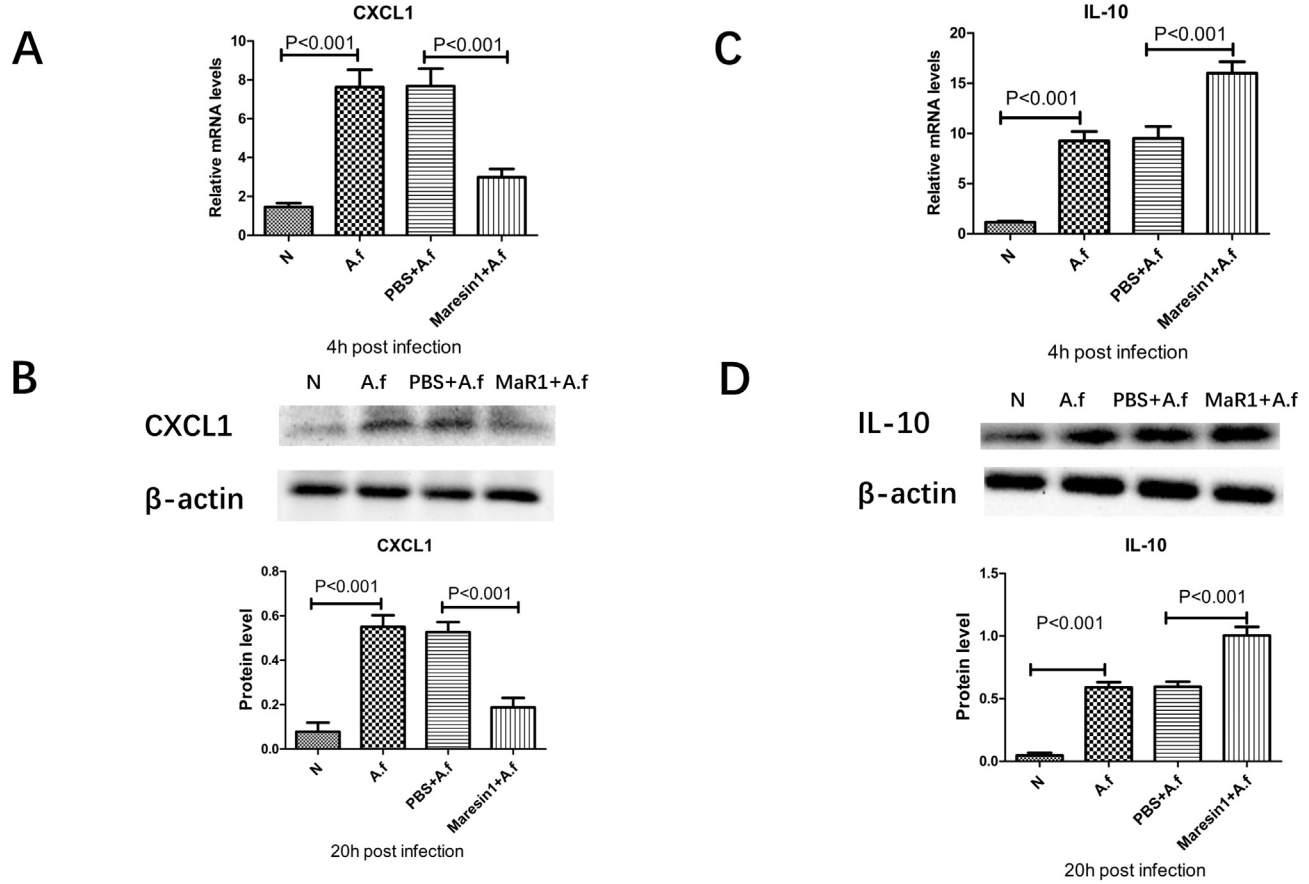

Fig. 4. (A–D) Maresin1 regulates IL-10 and CXCL1 expression in *A. fumigatus* stimulated RAW264.7 cells. Pretreatment with maresin1 reduced the mRNA levels (A) and protein levels (B) of the chemokine CXCL1 compared with the PBS group in *A. fumigatus* stimulated RAW264.7 cells. Additionally, mRNA levels (C) and protein levels (D) of IL-10 were enhanced after maresin1 pretreatment in RAW264.7 cells stimulated by *A. fumigatus* when compared with the PBS group.

recruitment of neutrophils into the corneal stroma [26]. Previous studies have established that a key role of maresin1 in accelerating resolution of inflammation is through reducing CXCL1. Li et al. [19] found that RvE1, which is an EPA derived molecule, possesses the ability to inhibit CXCL/KC in herpes simplex keratitis. Our in vivo and in vitro studies showed that maresin1 inhibits the production of CXCL1 in response to *A. fumigatus* infection.

Another important finding in our work with maresin1 is the

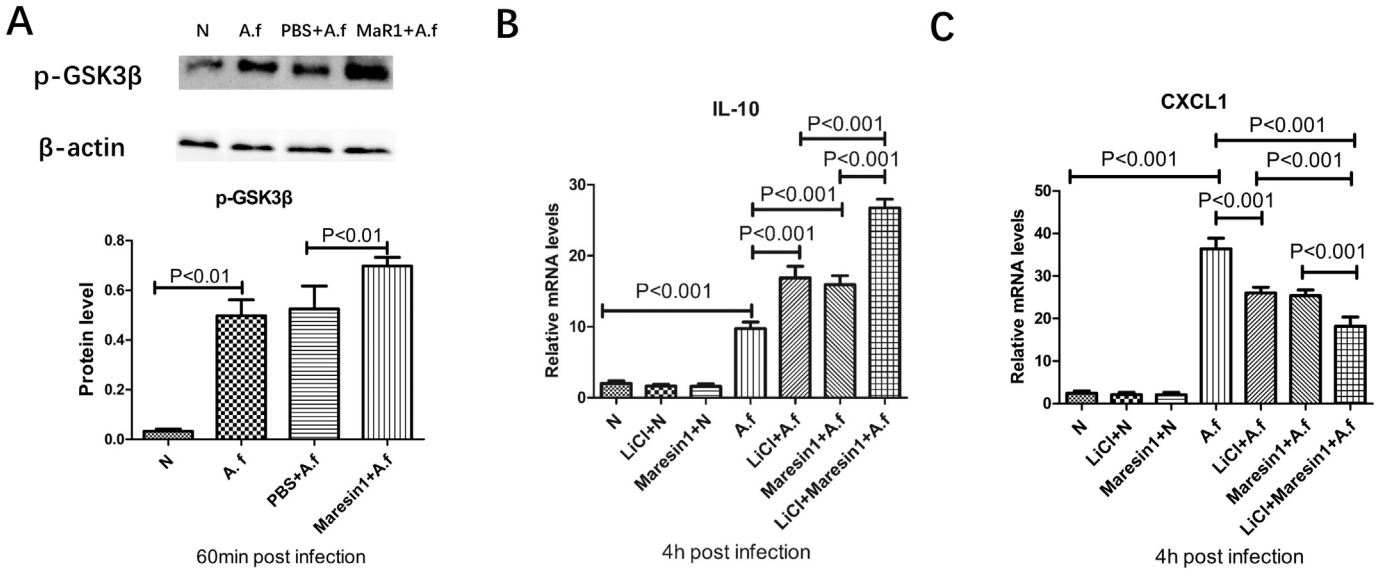

Fig. 5. (A–C) Maresin1 regulates CXCL1 and IL-10 by increasing p-GSK3β levels to inhibit GSK3β activity in RAW264.7 cells stimulated by *A. fumigatus*. Maresin1 pretreatment increased p-GSK3β ($p < 0.01$) levels in *A. fumigatus* stimulated RAW264.7 cells at 60 min (A). Additionally, inhibition of GSK3β by 20 nM LiCl reduced CXCL1 mRNA levels, which were further reduced by the combination of maresin1 and LiCl (B). What's more, the mRNA level of anti-inflammatory cytokines IL-10 was further increased by combination of maresin1 and LiCl.

upregulation of IL-10. IL-10 is considered to be a major regulator of immune responses and has been demonstrated to attenuate FK lesions in numerous previous studies. As a Th2 -type cytokine, it functions to regulate the anti-inflammatory response and limit unnecessary injury [27,28]. For example, IL-10 can inhibit the production and release of many cytokines such as IL-1 and TNF-a, and can suppress a Th1 type immune response [28]. Moreover, IL-10 is essential for integrity of tissue epithelial layers and participates in tissue healing after infection [29]. A number of experiments suggest that immune cells such as macrophages are responsible for producing various active proinflammatory cytokines during corneal inflammation, including IL-1β and TNF-a, as well as the anti-inflammatory cytokine IL-10 [30]. Other studies show that DHA-derived PAFUs like RvE1 can inhibit bacteria- and LPS-induced IL-1β, TNF-α, and CXCL1 production, and can enhance IL-10 levels during inflammation. Results of our current in vitro and in vivo studies support an anti-inflammatory role for maresin1 as we found that treatment with maresin1 contributes to an enhancement of anti-inflammatory cytokines such as IL-10, which could be responsible for the protective role of maresin1.

Glycogen synthase kinase-3β, a serine/threonine protein kinase, is a complex regulator of numerous cellular functions [31]. Previous studies demonstrated that GSK3β is a crucial mediator in the regulation of inflammation by regulating production of pro- and anti-inflammatory cytokines [32]. Inhibiting GSK3β reduced levels of proinflammatory cytokines like IL-6 and TNF-a but increased the anti-inflammatory cytokine IL-10 [33]. Moreover, phosphorylation of GSK3β resulted in GSK3β inactivation, and related downstream proinflammatory cytokines were reduced after LPS stimulation [34]. Our data demonstrate that maresin1 increases p-GSK3β levels in *A. fumigatus* stimulated RAW264.7 cells at 60 min. Furthermore, the ability of maresin1 to reduce CXCL-1 and increase the anti-inflammatory cytokine IL-10 was further augmented by LiCl, which can also inhibit GSK3β activity.

In summary, our studies demonstrate that treatment with maresin1 inhibits corneal inflammation induced by *A. fumigatus*. Furthermore, maresin1 regulates the chemokine CXCL-1, the cytokine IL-10 and neutrophil recruitment in *A. fumigatus* keratitis. Additionally, the regulation of CXCL1 and IL-10 by maresin1 is mediated by the inactivation of GSK3β by increasing p-GSK3β in RAW264.7 cells stimulated by *A. fumigatus*. FK results in chronic inflammation and eventually causes corneal perforation. In our reported studies, administration of maresin1 reduced disease lesions and inflammatory response, thereby demonstrating a beneficial role of maresin1 in FK. Taken together, maresin1 may represent a novel endogenous protective unsaturated fatty acid in *A. fumigatus* keratitis.

Fundings

This study was supported by the National Natural Science Foundation of China (81170825, 81300730, 81470609). The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

References

[1] L.T. Hu, Z.D. Du, G.Q. Zhao, N. Jiang, J. Lin, Q. Wang, et al., Role of TREM-1 in response to Aspergillus fumigatus infection in corneal epithelial cells, Int. Immunopharmacol. 23 (1) (2014) 288–293.
[2] Y. Niu, G. Zhao, C. Li, J. Lin, N. Jiang, C. Che, 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. 59 (1) (2018) 166–175.
[3] N. Sharma, J. Chacko, T. Velpandian, J.S. Titiyal, R. Sinha, G. Satpathy, et al., Comparative evaluation of topical versus intrastromal voriconazole as an adjunct to natamycin in recalcitrant fungal keratitis, Ophthalmology 120 (4) (2013) 677–681.
[4] S. Mahmoudi, A. Masoomi, K. Ahmadikia, S.A. Tabatabaei, M. Soleimani, S. Rezaie, et al., Fungal keratitis: an overview of clinical and laboratory aspects, Mycoses 61 (12) (2018) 916–930.
[5] A.J. Wolf, D.M. Underhill, Peptidoglycan recognition by the innate immune system, Nat. Rev. Immunol. 18 (4) (2018) 243–254.
[6] J. Song, Y.F. Huang, W.J. Zhang, X.F. Chen, Y.M. Guo, Ocular diseases: immunological and molecular mechanisms, Int. J. Ophthalmol. 9 (5) (2016) 780–788.
[7] C.N. Serhan, Pro-resolving lipid mediators are leads for resolution physiology, Nature 510 (7503) (2014) 92–101.
[8] C.N. Serhan, Discovery of specialized pro-resolving mediators marks the dawn of resolution physiology and pharmacology, Mol. Asp. Med. 58 (2017) 1–11.
[9] C.N. Serhan, R. Yang, K. Martinod, K. Kasuga, P.S. Pillai, T.F. Porter, et al., Maresins: novel macrophage mediators with potent antiinflammatory and proresolving actions, J. Exp. Med. 206 (1) (2009) 15–23.
[10] C.W. Wang, R.A. Colas, J. Dalli, H.H. Arnardottir, D. Nguyen, H. Hasturk, et al., Maresin 1 biosynthesis and proresolving anti-infective functions with human-localized aggressive periodontitis leukocytes, Infect. Immun. 84 (3) (2015) 658–665.
[11] T.W. Jung, H.S. Park, G.H. Choi, D. Kim, S.H. Ahn, D.S. Kim, et al., Maresin 1 attenuates pro-inflammatory reactions and ER stress in HUVECs via PPARalpha-mediated pathway, Mol. Cell. Biochem. 448 (1-2) (2018) 335–347.
[12] R. Marcon, A.F. Bento, R.C. Dutra, M.A. Bicca, D.F. Leite, J.B. Calixto, Maresin 1, a proresolving lipid mediator derived from omega-3 polyunsaturated fatty acids, exerts protective actions in murine models of colitis, J. Immunol. 191 (8) (2013) 4288–4298.
[13] H. Seki, K. Fukunaga, M. Arita, H. Arai, H. Nakanishi, R. Taguchi, et al., The anti-inflammatory and proresolving mediator resolvin E1 protects mice from bacterial pneumonia and acute lung injury, J. Immunol. 184 (2) (2009) 836–843.
[14] D. El Kebir, P. Gjorstrup, J.G. Filep, Resolvin E1 promotes phagocytosis-induced neutrophil apoptosis and accelerates resolution of pulmonary inflammation, Proc. Natl. Acad. Sci. U. S. A. 109 (37) (2012) 14983–14988.
[15] R.P. Flesher, C. Herbert, R.K. Kumar, Resolvin E1 promotes resolution of inflammation in a mouse model of an acute exacerbation of allergic asthma, Clin. Sci. (Lond.) 126 (11) (2014) 805–814.
[16] C. Che, C. Li, J. Lin, J. Zhang, N. Jiang, K. Yuan, et al., Wnt5a contributes to dectin-1 and LOX-1 induced host inflammatory response signature in Aspergillus fumigatus keratitis, Cell. Signal. 52 (2018) 103–111.
[17] N.K. Rajasagi, S. Bhela, S.K. Varanasi, B.T. Rouse, Frontline Science: aspirin-triggered resolvin D1 controls herpes simplex virus-induced corneal immunopathology, J. Leukoc. Biol. 102 (5) (2017) 1159–1171.
[18] N.K. Rajasagi, P.B. Reddy, A. Suryawanshi, S. Mulik, P. Gjorstrup, B.T. Rouse, Controlling herpes simplex virus-induced ocular inflammatory lesions with the lipid-derived mediator resolvin E1, J. Immunol. 186 (3) (2011) 1735–1746.
[19] R. Li, Y. Wang, Z. Ma, M. Ma, D. Wang, G. Xie, et al., Maresin 1 mitigates inflammatory response and protects mice from sepsis, Mediat. Inflamm. 2016 (2016) 3798465.
[20] G. Zhao, M. Hu, C. Li, J. Lee, K. Yuan, G. Zhu, et al., Osteopontin contributes to effective neutrophil recruitment, IL-1beta production and apoptosis in Aspergillus fumigatus keratitis, Immunol. Cell Biol. 96 (4) (2018) 401–412.
[21] J. Wang, Neutrophils in tissue injury and repair, Cell Tissue Res. 371 (3) (2018) 531–539.
[22] A. Schreiber, A. Rousselle, J.U. Becker, A. von Massenhausen, A. Linkermann, R. Kettritz, Necroptosis controls NET generation and mediates complement activation, endothelial damage, and autoimmune vasculitis, Proc. Natl. Acad. Sci. U. S. A. 114 (45) (2017) E9618–E9625.
[23] C.M. Dickinson, B.W. LeBlanc, M.M. Edhi, D.S. Heffernan, M.H. Faridi, V. Gupta, et al., Leukadherin-1 ameliorates endothelial barrier damage mediated by neutrophils from critically ill patients, J. Intensive Care 6 (2018) 19.
[24] J.W. Griffith, C.L. Sokol, A.D. Luster, Chemokines and chemokine receptors: positioning cells for host defense and immunity, Annu. Rev. Immunol. 32 (2014) 659–702.
[25] R.L. Silva, A.H. Lopes, R.M. Guimaraes, T.M. Cunha, CXCL1/CXCR2 signaling in pathological pain: role in peripheral and central sensitization, Neurobiol. Dis. 105 (2017) 109–116.
[26] A. Planaguma, T. Domenech, M. Pont, E. Calama, V. Garcia-Gonzalez, R. Lopez, et al., Combined anti CXC receptors 1 and 2 therapy is a promising anti-inflammatory treatment for respiratory diseases by reducing neutrophil migration and activation, Pulm. Pharmacol. Ther. 34 (2015) 37–45.
[27] L.D. Hazlett, X. Jiang, S.A. McClellan, IL-10 function, regulation, and in bacterial keratitis, J. Ocul. Pharmacol. Ther. 30 (5) (2014) 373–380.
[28] H. Ghasemi, T. Ghazanfari, R. Yaraee, P. Owlia, Z.M. Hassan, S. Faghihzadeh, Roles of IL-10 in ocular inflammations: a review, Ocul. Immunol. Inflamm. 20 (6) (2012) 406–418.
[29] T.L. Keadle, P.M. Stuart, Interleukin-10 (IL-10) ameliorates corneal disease in a mouse model of recurrent herpetic keratitis, Microb. Pathog. 38 (1) (2005) 13–21.
[30] D. Hos, F. Bucher, B. Regenfuss, M.L. Dreisow, F. Bock, L.M. Heindl, et al., IL-10 indirectly regulates corneal lymphangiogenesis and resolution of inflammation via macrophages, Am. J. Pathol. 186 (1) (2016) 159–171.
[31] E. Beurel, S.F. Grieco, R.S. Jope, Glycogen synthase kinase-3 (GSK3): regulation, actions, and diseases, Pharmacol. Ther. 148 (2015) 114–131.
[32] Q. Cao, A. Karthikeyan, S.T. Dheen, C. Kaur, E.A. Ling, Production of proinflammatory mediators in activated microglia is synergistically regulated by Notch-1, glycogen synthase kinase (GSK-3beta) and NF-kappaB/p65 signalling, PLoS One 12 (10) (2017) e0186764.
[33] H. Wang, A. Kumar, R.J. Lamont, D.A. Scott, GSK3beta and the control of infectious bacterial diseases, Trends Microbiol. 22 (4) (2014) 208–217.
[34] Z. Gu, G.J. Lamont, R.J. Lamont, S.M. Uriarte, H. Wang, D.A. Scott, Resolvin D1, resolvin D2 and maresin 1 activate the GSK3β anti-inflammatory axis in TLR4-engaged human monocytes, Innate Immun. 22 (3) (2016) 186–195.