• Review Article •

Immunologic mechanism of fungal keratitis

Rui-Bo Yang, Li-Ping Wu, Xiao-Xiao Lu, Chen Zhang, Hui Liu, Yue Huang, Zhe Jia, Yi-Chen Gao, Shao-Zhen Zhao

Tianjin Key Laboratory of Retinal Functions and Diseases, Tianjin Branch of National Clinical Research Center for Ocular Disease, Eye Institute and School of Optometry, Tianjin Medical University Eye Hospital, Tianjin 300384, China

Co-first authors: Rui-Bo Yang and Li-Ping Wu

Correspondence to: Shao-Zhen Zhao. Tianjin Medical University Eye Hospital, 251 Fukang Road, Xiqing District, Tianjin 300384, China. zhaosz1997@sina.com

Received: 2020-12-15 Accepted: 2021-01-14

Abstract

● Fungal keratitis (FK) is a refractory disease that poses a serious threat to vision, with common risk factors like eye trauma, contact lens wearing, topical corticosteroids and antibiotic abuse. Nowadays, topical and systemic anti-fungal drugs and ocular surgeries are still the main therapeutic modalities. However, the pathogenesis of FK, especially the immunologic mechanism within it, has not yet been deeply clarified. A better understanding of the pathogenesis of FK is imperative for more effective therapies and prognosis. Meanwhile, the immune protection strategies are also urgently required to manage FK. This review highlights recent advances in the immunologic mechanism in the pathogenesis of FK, in hope of providing valuable reference information for more effective anti-fungal treatment.

● **KEYWORDS:** fungal keratitis; innate immunity; adaptive immunity; pattern recognition receptors; immune cells

DOI:10.18240/ijo.2021.07.20

Citation: Yang RB, Wu LP, Lu XX, Zhang C, Liu H, Huang Y, Jia Z, Gao YC, Zhao SZ. Immunologic mechanism of fungal keratitis. *Int J Ophthalmol* 2021;14(7):1100-1106

INTRODUCTION

Fungal keratitis (FK), an intractable and sight-threatening disease, is an opportunistic infection of the cornea with a high rate of blindness[1]. In general, FK is often caused by the following species of fungi: *Candida albicans*, *Fusarium*, *Aspergillus*, *etc*. The incidence of FK has been increasing in recent years due to eye injury, long-term use of antibiotic or corticosteroid, contact lens wearing, and abnormal body immunity. Antifungal eye drops are still the main treatment of FK, which include natamycin, amphotericin B, if these medicines are not effective, to save vision and reconstruct eyeball the corneal transplantation must be performed. Even so, in some case, the disease still out of control. In order to administrate the intractable infectious keratitis better, we need to know how the disease starts and the progresses in the immunologic mechanism. In FK, the clinical prognosis largely depends on both pathogenic aggressiveness and host immune defense[2]. The defense ability of the host can be divided into physical barrier defense (tight junctions between the layered epithelial cells provide the major physical barrier defense[3]) and immune defense. This review will focus on the mechanism and research progress of host immune defense capability (including innate immunity and adaptive immunity) in the pathogenesis of FK.

Innate Immunity By providing a physical and immunological barrier, the ocular surface plays an important role in protecting visual organ[4]. There exists a large number of anti-inflammatory and bactericidal substances on the ocular surface, such as secretory IgA[5], mucin glycoproteins[6] and antimicrobial peptide (AMPs), which mainly exist in tear fluid to inhibit or kill microbes[7]. Cornea, serves as a barrier to protect intraocular tissue from damage, is crucial to the innate immune responses in defending against fungal infection. Previous studies showed the human corneal epithelium expressed at least 2 groups of AMPs to resistant to fungi, include LL-37, the only cathelicidin protein that human express, and human β-defensins (hBD1-4)[7].

Chitin, β-glucan and mannan are conserved in the fungal cell, and act as pathogen-associated molecular patterns (PAMPs) in the immune system of the host[8]. In the process of innate immune responses, the PAMPs will be recognize by pattern recognition receptors (PRRs), which are expressed on a wide variety of cell types, including macrophages, dendritic cells, neutrophils, then to mediate adhesion, absorption and eradication of pathogens[9-10]. PRRs that participate in the immune response mainly include C-type lectin receptors (CLRs), Toll-like receptors (TLRs), nucleotide-binding oligomerization domain-like receptors (NLRs) and scavenger receptors (SRs)[11].

Pattern recognition receptors

C-type lectin receptors C-type lectins are a class of protein superfamilies containing Ca^{2+}-dependent carbohydrate recognition domain (CRD), and serve as a kind of important PRRs in innate immune system[12].

β-glucan accounts for approximately 50% of the fungal cell wall dry weight[13] and can be specifically recognized by C-type lectin-1 (Dectin-1). Similarly, curdlan, a pure linear β-(1,3)-glucan has been identified as a selective Dectin-1 agonist. A research from Xu *et al*[14] demonstrated that curdlan pretreatment can activate Dectin-1, increase the level of pro-inflammatory cytokines and enhance the innate immune response to hyphae in human corneal epithelial cells (HCECs). In order to explore the role of Dectin-1 on recruiting inflammatory cells during the early period of FK, Xu *et al*[14] pretreated rat cornea with 20 mg/mL laminarin eye drop (Dectin-1 inhibitor) for 2h, then made rat FK model. They found that Dectin-1 might work through interleukin (IL)-1β, IL-6, C-C motif chemokine ligand 2 (CCL2), C-X-C motif chemokine ligand 1 (CXCL1), CXCL2 to recruit neutrophils and macrophages to participate in anti-fungal immunity in the early stage of rat FK. Triggering receptor in myeloid cells-1 (TREM-1) expressed by monocytes and polymorphonuclear leucocytes (PMNs)[15], is reported to be a critical inflammation amplifier[9]. Zhong *et al*[16] proposed that TREM-1 had a synergistic effect with Dectin-1. They demonstrated that blocking TREM-1 and Dectin-1 simultaneously would downregulate the inflammatory responses more effectively than blocking each of them alone. The caspase recruitment of domain protein 9 (CARD-9) is a key transducer of Dectin-1 signaling pathway and can control Dectin-1-mediated myeloid cell activation, cytokine production, and innate antifungal immunity[17]. Thus, Dectin-1-CARD-9 pathway is thought to be a crucial component of immune responses to fungi[18].

Macrophage-inducible C-type lectin (Mincle) is a newfound C-type lectin receptor, which is expressed on myeloid cells and neutrophils, especially on macrophages, dendritic cells, and B cells. Mincle can be significantly upregulated by different inflammatory signal stimulation, even though its expression is very low in steady-state condition[19]. Recent studies proved that Mincle pathway participates in the expression of tumor necrosis factor (TNF)-α, IL-1β, IL-10, and CCL3 during the progress of FK. As a PRR, Mincle may play a key part during the early period of innate immune response to the fungi[20]. Yu *et al*[21] investigate the details that how Mincle influences inflammation in *Aspergillus fumigatus* (AF) induced FK mice, the mice were subconjunctivally injected by Mincle agonist trehalose-6,6-dibehenate (TDB group), goat anti-mouse Mincle neutralizing antibody (Mincle Ab group), 0.5% dimethylsulfoxide (DMSO) or goat IgG (control group) respectively before the cornea were infected by AF. Cytokines (IL-1β, TNF-α, IL-6), chemokines (CXCL-1 and -2, which are important in neutrophils recruitment), neutrophils infiltration were observed, and the levels of nitric oxide (NO) generated by corneas were also tested. The results showed that the levels of Mincle mRNA and protein were higher in infected corneas compared with normal corneas. When pretreated with TDB, the levels of IL-1β, TNF-α, IL-6, CXCL1 and macrophage inflammatory protein 2 (MIP-2) in infected corneas were dramatically higher than control group while the expression of above factors were down-regulated in Mincle Ab group, coinciding with neutrophils infiltration in corneas. As for the concentration of NO, it was promoted in TDB group and decreased in Mincle Ab group when comparing with control group. So, they summarized that Mincle participated in the innate immune response by enhancing inflammation and mediated cytotoxicity by regulating the formation of NO.

Surfactant protein D (SP-D) is also a member of the C-type lectin protein family, which contributes to pulmonary host defense and inflammation regulation[22]. Ni *et al*[23-24] found that SP-D, existing in human tear fluid and cornea epithelium, could protect cornea from *Pseudomonas aeruginosa* infection. Given that, Wu *et al*[25] investigated the interaction of SP-D and Toll-like receptor-4 (TLR4) signaling pathway in HCECs when stimulated by inactive hyphae of AF. They found that TLR4 could activate SP-D and be inhibited by SP-D in turn. During the process of fungal infection, SP-D also plays a role in immunosuppression, and SP-D's suppression to inflammatory cytokines can be achieved through TLR4 signaling pathway.

Toll-like receptors TLRs are transmembrane receptors. After recognizing the PAMPs, TLRs will activate nuclear factor-kappa B (NF-κB) through MyD88-dependent or non-MyD88-dependent pathway, then promote the expression of TNF-α, IL-1β, IL-6, IL-8, INF-γ and initiate innate immune responses.

Until now, 10 functional human TLRs have been identified[26], in which, TLR2 and TLR4 on corneal epithelial cells, keratocytes, and leukocytes can recognize fungi through NF-κB and made immune cells produce proinflammatory cytokines to contribute to the pathogenesis of FK[27-28]. Previous studies have shown that corneal epithelial cells participate in the host response against pathogens through the recognition of TLRs by promoting the expression of inflammatory cytokines, recruiting inflammatory cells to the infection site and producing antimicrobial molecules. However, exaggerated expression of inflammatory components has devastating effects on the host. Guo *et al*[29] applied TLR2 small interfering RNA (siRNA) subconjunctivally injected to investigate the effect of TLR2 on AF induced keratitis in rats, which revealed that TLR2 siRNA could improve the prognosis of FK and prevent corneal perforation by suppressing PMN infiltration,

down-regulating the inflammatory cytokines significantly and reducing the fungal burden. Thymic stromal lymphopoietin (TSLP), an interleukin 7 (IL-7)-like four helix bundle cytokine, playing various roles in the regulation of immune responses, is expressed primarily by epithelial cells and keratocytes[30] and binds to its unique thymic stromal lymphopoietin receptor (TSLPR) to transmit signals in cells[31]. A research from Dai *et al*[32] investigate the correlation between TSLP and TLR2, TLR4 in innate immunity induced by AF infection, which showed that TSLP promoted the expression of TLR2/TLR4 and their downstream inflammatory cytokines (IL-6 and IL-8) to modulate the severity of fungal corneal infection.

Nucleotide-binding oligomerization domain-like receptors Different from TLRs, NLRs are a kind of cytoplasmic receptors which act as intracellular receptors responding to microbes and microbial components[33]. Two members of NLR family, NOD-like receptor 1 (NOD-1) and NOD-like receptor 2 (NOD-2), are in charge of recognizing bacterial peptidoglycan and activating immune responses *via* NF-κB and mitogen-activated protein kinase (MAPK)[34]. Zhang *et al*[35] found the expression of NOD-1 and its downstream molecules such as receptor-interacting protein 2 (RIP2) and NF-κB p65 were significantly elevated in HCECs that were inoculated by heat-inactivated AF conidia beforehand, while the secretion of IL-6, IL-8, TNF-α, antibacterial peptides hBD2 and LL37 were apparently reduced if NOD-1 was knocked down. The results suggested that NOD1 signaling pathways were essential for HCECs in the recognition of AF and played a vital role in the innate immune responses in FK. Many studies also revealed that NOD-2 could increase the production of pro-inflammatory cytokines then influence the innate immunity[34,36-37]. Wu *et al*[25] demonstrated that the increased expression of NOD-2 caused by AF conidia occurred in part through a TLR2-dependent pathway, and suggested there may exist some complex interactions between TLR2 and NOD-2 in HCECs inflammatory response against AF infection.

Scavenger receptors The SRs family, which also play critical roles in host defense against various pathogens. Lectin-like oxidized low-density lipoprotein receptor 1 (LOX-1), a lectin-like 52-kD type II membrane receptor for oxidized low-density lipoproteins, belongs to the SRs family[11] and also structurally belongs to CLRs[38]. LOX-1 is mainly expressed on endothelial cells, macrophages, neutrophils, vascular smooth muscle cells, and platelets. A few studies from Li *et al*[10] showed LOX-1 mRNA significantly increased in AF infected mice corneas, and LOX-1 inhibition could reduce the inflammatory response and rebalance the inflammatory response of FK[38]. The studies[39] also revealed that LOX-1 and TLR4 interacted with each other in AF keratitis of mice, and controlled inflammation through regulating the generation of reactive oxygen species (ROS) and IL-1β.

Innate Immunocyte Once the PAMPs are recognized by PRRs, the production of pro-inflammatory cytokines will be initiated and enhanced through MyD88/NF-κB signaling pathway or Dectin-1-CARD9 (the caspase recruitment domain protein 9) signaling pathway, along with the phagocytosis of macrophages and infiltration of neutrophils being activated for the aim of fungi clearance.

Macrophages Macrophages, as the first-line defense cells for the host's immune system[40], play an important role in various types of corneal inflammation. It is difficult to find macrophages in normal corneal tissues, only after the cornea is damaged, will macrophages can be detected in the limbus or central cornea to participate in the local inflammatory response. It has been demonstrated that macrophages could inhibit spore germination and kill spores or hyphae of fungi *in vivo* and *in vitro*[41-42]. Hu *et al*[43] investigated the potential roles of macrophages in FK. They injected liposomes containing dichloromethylene diphosphonate (Cl2MDP-LIP; after phagocytosis and disruption of the phospholipid bilayers, the Cl2MDP can be released into the cytoplasm to make macrophage apoptosis) to BALB/c mice which suffer from *Fusarium solani* and *Candida albicans* keratitis respectively to deplete macrophages. Compared with the controls [received liposomes containing phosphate-buffered saline (PBS-LIP)], the fungal clearance and PMNs infiltration were lower in the Cl2MDP-LIP group, which resulted in an exacerbation of FK. The mechanism maybe that the depletion of local macrophages accompanied by TLR4 deficiency reduce the production of corneal proinflammatory cytokines and result in the delay of fungal clearance and corneal destruction. In addition, Hu *et al*[44] also investigated the potential role of activated macrophages in *Fusarium solani* induced FK mice model. They injected latex drops into the superficial stroma of the cornea to activate macrophages and took PBS-treated group as the control group. The results showed that the speed of fungal clearance and PMN infiltration were higher in the latex bead-treated corneas than PBS-treated corneas, inducible nitric oxide synthase (iNOS) and migration inhibitory factor (MIF) were also markedly increased in latex bead-treated group. However, the latex drops group exhibited excessive inflammation and corneal perforation. So, they speculated that although the activated macrophages have a great killing effect on fungi, the enhancement of PMN infiltration and pro-inflammatory cytokines expression provoke an excessive immune responses and result in corneal perforation.

Neutrophils Same as macrophages, neutrophils also belong to the first -line defender cells of host immunity. They can defend the host against the invasion of pathogens by phagocytosis, degranulation, production of ROS, antimicrobial peptides, and extrusion of neutrophil extracellular traps (NETs)[45-47]. During the inflammatory process of FK, the ROS-induced oxidative

injury can damage the tissue *via* p38MAPK pathway[48]. Hua *et al*[49] found that inhibition of p38MAPK may decrease ROS production of neutrophils and alleviate the tissue damage caused by oxidative stress. Being composed of DNA, histones, and neutrophil elastase, NETs is a kind of web-like structures released extracellularly from activated neutrophils during infection[50]. NETs are capable of netting high concentration of antimicrobial peptide can to eradicate microbes more effectively. Jin *et al*[51] evaluated the significance of NETs in the process of FK, they demonstrated that a more NETs formation may lead to a better prognosis. In another relative study, Alasmari[47] observed NETs formation in the cornea lesion after infected by *Candida albicans*, they treated the FK mice with 0.1% dexamethasone (DXM) drops 3 times each day for 3d, and took saline treated group as control group. Compared with control group, the DXM treatment group had a higher clinical score, a deeper infiltration of fungi, but a fewer number of NETs and neutrophils. They proposed that glucocorticoids made FK deteriorative not only by enhancing the fungal aggressiveness and reducing the infiltration of neutrophils, but also by inhibiting the extrusion of NETs[52]. And these findings may provide us a new view of glucocorticoids impact on FK.

Mast cells Until now, few studies have been reported on the role of MCs in antifungal defense[53]. Research from Xie *et al*[54] explored the functions and mechanisms of MCs during FK. They treated FK mice with cromolyn sodium (MC stabilizer) to block MC degranulation, suppress vascular dilation and permeability, and reduce neutrophil infiltration with lower intercellular cell adhesion molecule-1 (ICAM-1) levels. Compared with untreated FK mice, the blocked mice manifested prolonged disease course and more severe pathological damage. And they proposed a novel viewpoint that MCs may play a pivotal role in protecting cornea against the damage of fungal infection.

Adaptive Immunity It is well known that activated innate immunity will subsequently lead to effective adaptive immunity. However, in the field of ocular infection of fungi, the adaptive immune response has been overlooked for a long time.

Zhang *et al*[41] firstly reported the adaptive immune response induced by FK. They used pre-fungal keratitis mice model (pre-FK, intrastromal injection of *Candida albicans* spores), pre-pulmonary infection mice model (pre-PI, intranasal inhalation *Candida albicans* of spores), and pre-active immunization mice model (pre-AI, subcutaneous injection of heat inactivated spores) respectively as primary *Candida albicans* infection diseases. All the primed mice were left untreated until 8wk, then intrastromal reinfected with *Candida albicans* spore to induce secondary *Candida albicans* keratitis (CaK). When mice corneas reinfected by *Candida albicans* spore, they developed less severe FK than previous infection, and the corneas regained transparency more quickly than before. The CaK in pre-PI mice exhibited milder FK and quicker healing than primary CaK infection. Compared with control and pre-PI groups, the pre-AI group induced by heat inactivated spores manifested a similar but stronger vaccination effect, the secondary CaK in pre-AI group accompanied by lower disease scoring and quicker recovery process. The serum was collected after the induction of secondary CaK for the measurement of IgA, IgG, IL-4, and IFN-γ. The results showed the mice pretreated by *Candida albicans* produced more IgG and IgA in serum, with more immunoglobulin deposition and lymphocytes infiltration existing in corneas of secondary CaK. So, they proposed that priming the murine immune systems by infecting other organs with live spores or by vaccination with inactivated spores can prevent the cornea against the same fungi infection. A recent study from Wang *et al*[55] showed that HCECs being stimulated with AF hyphae would expressed exaggerated TSLP which could induce the activation of CD4+ T cells, CD8+ T cells, B cells, increase the proliferation of peripheral blood mononuclear cells (PBMCs) significantly, meanwhile, enhance the production of T-helper (Th) 2 type cytokines (IL-4 and IL-13) and IgG. They summarized TSLP that were produced by AF infected HCECs could act as a key molecule in adaptive immune responses of FK *via* skewing Th2 differentiation and promoting humoral immunity. Hu *et al*[43-44] also proposed that macrophages played a role in the differentiation of Th1 cells and Th2 cells during the adaptive immunity response of FK. They speculated macrophages depletion induced a shift from a Th1- to a Th2-dominated immune responses, leading to downregulation of the immune response. Moreover, macrophage activation can disrupt the balance between Th1 and Th2 responses, which induces a much stronger and more persistent Th1 response, eventually resulting in necrosis and perforation of the cornea.

Others In addition to the immune cells and cytokines mentioned above, Other cell products, such as vasoactive intestinal peptide (VIP), IFN-stimulated gene of 15 kDa (ISG15), Maresin1 (MaR1), indoleamine 2,3 dioxygenase (IDO) also play vital roles in the immune process of FK.

VIP, a type of neurotransmitter or neuropeptide, exists in the central and enteric nervous systems and plays anti-inflammatory role in immune responses[56]. VIP is produced by immune cells: such as mast cells, granulocytes, and lymphocytes. Previous studies proposed VIP acts as a regulator to rebalance the expression of pro- or anti-inflammatory factors[57]. It has been reported that VIP could destroy the membrane structure of pathogens and eliminate various pathogens including fungi[48]. Li *et al*[58] investigated the protective responses of VIP in AF induced FK. They used C57BL/6 and BALB/c mice as the FK model. C57BL/6 mice were pretreated with recombinant (r)VIP (intraperitoneal injection) to explore whether exogenous VIP led to better

disease prognosis, while BALB/c mice were pretreated with VIP antagonist, and then infected by AF. The results demonstrated that rVIP treatment lowered clinical scores of C57BL/6 mice, along with less corneal PMN infiltrate and IL-1β, TNF-α expression and increased production of anti-inflammatory mediator IL-10. So VIP was considered as an anti-inflammatory factor in AF induced FK.

ISG15, a di-ubiquitin-like protein, is crucial for controlling bacteria and virus infection[59]. However, there are few studies on the role of ISG15 in fungal infection. A study of Dubey *et al*[8] showed ISG15 acted as an immunomodulator in cornea and played a critical role in controlling FK. They infected C57BL/6 (B6) mice corneas with *Candida albicans* (*in vivo*) and treated HCECs with heat-inactivated *Candida albicans* (*in vitro*). Flagellin was applied as an immunostimulant to activate innate immunity. Whether *in vitro* or *in vivo*, flagellin could augment the expression of ISG15 induced by *Candida albicans*. B6 mice were subconjunctival injected with 10 μmol/L ISG15 siRNA and 2 μL recombinant ISG15 respectively to investigate the role of ISG15 in *Candida albicans* induced FK. The experimental results showed ISG15 knockdown increased the severity of FK (increased corneal fungal burden) and abolished flagellin-induced protection responses. Meanwhile, PMN infiltration and expression of CXCL2 also increased. In contrast, injection of recombinant ISG15 dramatically much reduced the severity of FK, including decreased fungal burden and PMN infiltration. Other studies suggested ISG15 could control the corneal infection through down regulating IL-1β and IFN-γ, inhibiting inflammatory response, and inducing CXCL10 expression[60-61].

MaR1 is a pro-inflammatory degradation product derived from docosahexaenoic acid (DHA) and synthesized by macrophages[62]. Li *et al*'s[61] research showed that MaR1 could play a protective role during the process of sepsis by reducing bacteria burden and inhibiting inflammation response. Tang *et al*[63] induced the mice FK by injecting AF to corneal stroma, then MaR1 was injected subconjunctival 2h later. In addition to that, topical administration of MaR1 was applied twice a day from day 1 to day 5. The level of inflammation cells and cytokine were assessed. The results showed that, MaR1 treatment could moderate the severity of FK by reducing the infiltration of neutrophils and the expression of CXCL1. MaR1 also enhanced the production of anti-inflammatory cytokines, such as IL-10.

IDO was found to be mainly expressed on macrophages, PMNs, activated dendritic cells[64], and epithelial cells. Jiang *et al*[65] observed the location and expression of IDO in AF induced FK mice. They tested the expression of IDO in the normal and infected corneas of mice. Immunofluorescence staining showed IDO was mainly expressed in epithelium and stroma in the AF infected corneal tissues. To further explored the function of IDO in the pathogenesis of FK[66], HCECs were added with 1 ng/mL 1-methyl tryptophan (1-MT, an inhibitor of IDO) for 10h before inactive AF spores stimulation. The result showed application of 1-MT significantly increased the expression of IL-1β and IL-6 compared with the simple AF stimulation group, which demonstrated that IDO overexpression took part in the progress of infection by modulating the pro-inflammatory cytokines and immune responses.

CONCLUSION

The host's antifungal process is complex. When fungi adhered to the damaged corneal tissue, the PAMPs (*e.g.*, chitin, β-glucan and mannan) located on the fungal cell wall can be recognized by PRRs (*e.g.* TLRs, CLRs, NLRs, SR) presenting on the innate immune cells of host. As a result, these immune cells release various types of cytokines, including interleukins, tumor necrosis factors, and chemokines, which promote the macrophages and neutrophils to migrate to the infection site and trigger subsequent inflammatory reactions. Meanwhile, other cellular products, such as VIP, MaR1, IDO also regulate the immune process. What we need to know is that the immune response of host is a double-edged sword. The release of inflammatory mediators is essential for the host to kill fungi, however excessive inflammation burst can also damage normal corneal tissue. Therefore, finding a balance between eliminating pathogenic fungi and reducing inflammation damage is a subject worthy of more in-depth study. In recent years, the researches on the pathogenesis of FK have got continuous progress. However, the current treatment strategy for FK is still relatively simple and less than optimal. The more thorough research on the pathogenic mechanism of FK, the more valuable advances we can get on its treatment. Hopefully, more direct and effective anti-fungal therapies would be applied in the clinic to protect more patients from blindness.

ACKNOWLEDGEMENTS

Foundations: Supported by the Natural Science Foundation of Tianjin (No.19JCYBJC25700); Clinical Key Discipline (Specialty) Construction Project of Tianjin (No. TJLCZDXKM002); Science and Technology Projects of Social Development of Tianjin Binhai New District (No. BHXQKJXM-SF-2018-05); Science and Technology Projects of Health Commission of Tianjin Binhai New District (No.2019BWKZ008).

Conflicts of Interest: Yang RB, None; **Wu LP,** None; **Lu XX,** None; **Zhang C,** None; **Liu H,** None; **Huang Y,** None; **Jia Z,** None; **Gao YC,** None; **Zhao SZ,** None.

REFERENCES

1 Hu LT, Du ZD, Zhao GQ, Jiang N, Lin J, Wang Q, Xu Q, Cong L, Qiu S. Role of TREM-1 in response to Aspergillus fumigatus infection in corneal epithelial cells. *Int Immunopharmacol* 2014;23(1):288-293.

2 Yuan XY, Wilhelmus KR. Toll-like receptors involved in the pathogenesis of experimental Candida albicans keratitis. *Invest Ophthalmol Vis Sci* 2010;51(4):2094-2100.

3 Ueta M, Kinoshita S. Innate immunity of the ocular surface. *Brain Res Bull* 2010;81(2-3):219-228.

4 Leong YY, Tong L. Barrier function in the ocular surface: from conventional paradigms to new opportunities. *Ocul Surf* 2015;13(2):103-109.

5 Yaginuma S, Akune Y, Shigeyasu C, Takano Y, Yamada M. Tear protein analysis in presumed congenital alacrima. *Clin Ophthalmol* 2018;12:2591-2595.

6 Fini ME, Jeong S, Gong H, Martinez-Carrasco R, Laver NMV, Hijikata M, Keicho N, Argüeso P. Membrane-associated mucins of the ocular surface: new genes, new protein functions and new biological roles in human and mouse. *Prog Retin Eye Res* 2020;75:100777.

7 Hua X, Yuan XY, Tang X, Li ZJ, Pflugfelder SC, Li DQ. Human corneal epithelial cells produce antimicrobial peptides LL-37 and β-defensins in response to heat-killed Candida albicans. *Ophthalmic Res* 2014;51(4):179-186.

8 Dubey LK, Moeller JB, Schlosser A, Sorensen GL, Holmskov U. Induction of innate immunity by Aspergillus fumigatus cell wall polysaccharides is enhanced by the composite presentation of chitin and beta-glucan. *Immunobiology* 2014;219(3):179-188.

9 Arts RJ, Joosten LA, van der Meer JW, Netea MG. TREM-1: intracellular signaling pathways and interaction with pattern recognition receptors. *J Leukoc Biol* 2013;93(2):209-215.

10 Li C, Zhao GQ, Che CY, Lin J, Li N, Hu LT, Jiang N, Liu Y. The role of LOX-1 in innate immunity to aspergillus fumigatus in corneal epithelial cells. *Invest Ophthalmol Vis Sci* 2015;56(6):3593-3603.

11 Lee EJ, Brown BR, Vance EE, Snow PE, Silver PB, Heinrichs D, Lin X, Iwakura Y, Wells CA, Caspi RR, Rosenzweig HL. Mincle activation and the Syk/Card9 signaling axis are central to the development of autoimmune disease of the eye. *J Immunol* 2016;196(7):3148-3158.

12 Zelensky AN, Gready JE. The C-type lectin-like domain superfamily. *FEBS J* 2005;272(24):6179-6217.

13 Klis FM, Mol P, Hellingwerf K, Brul S. Dynamics of cell wall structure in Saccharomyces cerevisiae. *FEMS Microbiol Rev* 2002;26(3):239-256.

14 Xu Q, Zhao GQ, Lin J, Wang Q, Hu LT, Jiang Z. Role of Dectin-1 in the innate immune response of rat corneal epithelial cells to Aspergillus fumigatus. *BMC Ophthalmol* 2015;15:126.

15 Bouchon A, Dietrich J, Colonna M. Cutting edge: inflammatory responses can be triggered by TREM-1, a novel receptor expressed on neutrophils and monocytes. *J Immunol* 2000;164(10):4991-4995.

16 Zhong J, Huang WL, Deng QC, Wu MH, Jiang HL, Lin XL, Sun YF, Huang X, Yuan J. Inhibition of TREM-1 and dectin-1 alleviates the severity of fungal keratitis by modulating innate immune responses. *PLoS One* 2016;11(3):e0150114.

17 Gross O, Gewies A, Finger K, Schäfer M, Sparwasser T, Peschel C, Förster I, Ruland J. Card9 controls a non-TLR signalling pathway for innate anti-fungal immunity. *Nature* 2006;442(7103):651-656.

18 Xu LJ, Xie LX. Fusarium solani activates dectin-1 in experimentally induced keratomycosis. *Curr Med Sci* 2018;38(1):153-159.

19 Miyake Y, Ishikawa E, Ishikawa T, Yamasaki S. Self and nonself recognition through C-type lectin receptor, Mincle. *Self Nonself* 2010;1(4):310-313.

20 Zhao GQ, Xu Q, Lin J, Chen WJ, Cui TT, Hu LT, Jiang N. The role of Mincle in innate immune to fungal keratitis. *J Infect Dev Ctries* 2017;11(1):89-97.

21 Yu GR, Lin J, Zhang J, Che CY, Peng XD, Li C, Hu LT, Zhu GQ, He K, Zhao GQ. Mincle in the innate immune response of mice fungal keratitis. *Int J Ophthalmol* 2018;11(4):539-547.

22 Zhou Y, Ming JH, Li YM, Du XJ, Deng M, He B, Zhou JL, Wang GR, Liu SQ. Surfactant protein D attenuates nitric oxide-stimulated apoptosis in rat chondrocyte by suppressing p38 MAPK signaling. *Biochem Biophys Res Commun* 2018;495(1):526-532.

23 Ni MJ, Evans DJ, Hawgood S, Anders EM, Sack RA, Fleiszig SM. Surfactant protein D is present in human tear fluid and the cornea and inhibits epithelial cell invasion by Pseudomonas aeruginosa. *Infect Immun* 2005;73(4):2147-2156.

24 Ni MJ, Tam C, Verma A, Ramphal R, Hawgood S, Evans DJ, Fleiszig SM. Expression of surfactant protein D in human corneal epithelial cells is upregulated by Pseudomonas aeruginosa. *FEMS Immunol Med Microbiol* 2008;54(2):177-184.

25 Wu X, Zhao GQ, Lin J, Jiang N, Li C, Hu LT, Peng XD, Xu Q, Wang Q, Li H, Zhang Y. The production mechanism and immunosuppression effect of pulmonary surfactant protein D via toll like receptor 4 signaling pathway in human corneal epithelial cells during Aspergillus fumigatus infection. *Int Immunopharmacol* 2015;29(2):433-439.

26 Redfern RL, McDermott AM. Toll-like receptors in ocular surface disease. *Exp Eye Res* 2010;90(6):679-687.

27 Jin XM, Qin Q, Tu LL, Zhou XT, Lin Y, Qu J. Toll-like receptors (TLRs) expression and function in response to inactivate hyphae of Fusarium solani in immortalized human corneal epithelial cells. *Mol Vis* 2007;13:1953-1961.

28 Zhao J, Wu XY, Yu FS. Activation of Toll-like receptors 2 and 4 in Aspergillus fumigatus keratitis. *Innate Immun* 2009;15(3):155-168.

29 Guo H, Gao JL, Wu XY. Toll-like receptor 2 siRNA suppresses corneal inflammation and attenuates Aspergillus fumigatus keratitis in rats. *Immunol Cell Biol* 2012;90(3):352-357.

30 Liu YJ. TSLP in epithelial cell and dendritic cell cross talk. *Adv Immunol* 2009;101:1-25.

31 Wang R, Chen W, Ma ZF, Li LP, Chen X. M1/M2 macrophages and associated mechanisms in congenital bicuspid aortic valve stenosis. *Exp Ther Med* 2014;7(4):935-940.

32 Dai C, Wu J, Chen C, Wu X. Interactions of thymic stromal lymphopoietin with TLR2 and TLR4 regulate anti-fungal innate immunity in Aspergillus fumigatus-induced corneal infection. *Exp Eye Res* 2019;182:19-29.

33 Kim YK, Shin JS, Nahm MH. NOD-like receptors in infection, immunity, and diseases. *Yonsei Med J* 2016;57(1):5-14.

34 Jeong YJ, Kang MJ, Lee SJ, Kim CH, Kim JC, Kim TH, Kim DJ, Kim D, Núñez G, Park JH. Nod2 and Rip2 contribute to innate immune responses in mouse neutrophils. *Immunology* 2014;143(2):269-276.

35 Zhang YT, Wu JY, Xin ZT, Wu XY. Aspergillus fumigatus triggers innate immune response via NOD1 signaling in human corneal epithelial cells. *Exp Eye Res* 2014;127:170-178.

36 Jing HY, Fang LR, Wang D, Ding Z, Luo R, Chen HC, Xiao SB. Porcine reproductive and respiratory syndrome virus infection activates NOD2-RIP2 signal pathway in MARC-145 cells. *Virology* 2014;458-459:162-171.

37 Zhang HJ, Qu JM, Shao CZ, Zhang J, He LX, Yuan ZH. Aspergillus fumigatus conidia upregulates NOD2 protein expression both *in vitro* and *in vivo*. *Acta Pharmacol Sin* 2008;29(10):1202-1208.

38 He K, Yue LH, Zhao GQ, Li C, Lin J, Jiang N, Wang Q, Xu Q, Peng XD, Hu LT, Zhang J. The role of LOX-1 on innate immunity against Aspergillus keratitis in mice. *Int J Ophthalmol* 2016;9(9):1245-1250.

39 Gao XR, Zhao GQ, Li C, Lin J, Jiang N, Wang Q, Hu LT, Xu Q, Peng XD, He K, Zhu GQ. LOX-1 and TLR4 affect each other and regulate the generation of ROS in A. fumigatus keratitis. *Int Immunopharmacol* 2016;40:392-399.

40 Stafford JL, Neumann NF, Belosevic M. Macrophage-mediated innate host defense against protozoan parasites. *Crit Rev Microbiol* 2002;28(3):187-248.

41 Zhang HB, Chen H, Niu JY, Wang YQ, Xie LX. Role of adaptive immunity in the pathogenesis of Candida albicans keratitis. *Invest Ophthalmol Vis Sci* 2009;50(6):2653-2659.

42 Waldorf AR, Levitz SM, Diamond RD. *In vivo* bronchoalveolar macrophage defense against Rhizopus oryzae and Aspergillus fumigatus. *J Infect Dis* 1984;150(5):752-760.

43 Hu JZ, Wang Y, Xie LX. Potential role of macrophages in experimental keratomycosis. *Invest Ophthalmol Vis Sci* 2009;50(5):2087-2094.

44 Hu JZ, Hu YF, Chen SK, Dong CH, Zhang JJ, Li YL, Yang J, Han XL, Zhu XJ, Xu GX. Role of activated macrophages in experimental Fusarium solani keratitis. *Exp Eye Res* 2014;129:57-65.

45 Ashman RB. Detailed protocols and practical advice on a variety of modern approaches to the study of leucocytes and their products. Preface. *Methods Mol Biol* 2012;844:v.

46 Rosales C. Neutrophil: a cell with many roles in inflammation or several cell types? *Front Physiol* 2018;9:113.

47 Alasmari SZ. *In vivo* imaging of neutrophil extracellular traps (NETs): visualization methods and outcomes. *Biomed Res Int* 2020;2020:4192745.

48 Waetzig GH, Seegert D, Rosenstiel P, Nikolaus S, Schreiber S. p38 mitogen-activated protein kinase is activated and linked to TNF-alpha signaling in inflammatory bowel disease. *J Immunol* 2002;168(10):5342-5351.

49 Hua X, Chi W, Su L, Li J, Zhang ZD, Yuan XY. ROS-induced oxidative injury involved in pathogenesis of fungal keratitis *via* p38 MAPK activation. *Sci Rep* 2017;7(1):10421.

50 Brinkmann V, Reichard U, Goosmann C, Fauler B, Uhlemann Y, Weiss DS, Weinrauch Y, Zychlinsky A. Neutrophil extracellular traps kill bacteria. *Science* 2004;303(5663):1532-1535.

51 Jin XM, Zhao YY, Zhang F, Wan T, Fan FL, Xie X, Lin ZY. Neutrophil extracellular traps involvement in corneal fungal infection. *Mol Vis* 2016;22:944-952.

52 Fan FL, Huang XD, Yuan KL, Zhu BB, Zhao YY, Hu RJ, Wan T, Zhu L, Jin XM. Glucocorticoids may exacerbate fungal keratitis by increasing fungal aggressivity and inhibiting the formation of neutrophil extracellular traps. *Curr Eye Res* 2020;45(2):124-133.

53 Saluja R, Metz M, Maurer M. Role and relevance of mast cells in fungal infections. *Front Immunol* 2012;3:146.

54 Xie Y, Zhang H, Liu S, Chen G, He S, Li Z, Wang L. Mast cell activation protects cornea by promoting neutrophil infiltration *via* stimulating ICAM-1 and vascular dilation in fungal keratitis. *Sci Rep* 2018;8(1):8365.

55 Wang LP, Wang LY, Wu XY. Aspergillus fumigatus promotes T helper type 2 responses through thymic stromal lymphopoietin production by human corneal epithelial cells. *Clin Exp Ophthalmol* 2016;44(6):492-501.

56 Delgado M, Pozo D, Ganea D. The significance of vasoactive intestinal peptide in immunomodulation. *Pharmacol Rev* 2004;56(2):249-290.

57 Szliter EA, Lighvani S, Barrett RP, Hazlett LD. Vasoactive intestinal peptide balances pro- and anti-inflammatory cytokines in the Pseudomonas aeruginosa-infected cornea and protects against corneal perforation. *J Immunol* 2007;178(2):1105-1114.

58 Li C, Liu YY, Zhao GQ, Lin J, Che CY, Jiang N, Li N, Zhang J, He K, Peng XD. Role of vasoactive intestinal peptide in *Aspergillus fumigatus*-infected cornea. *Int J Ophthalmol* 2018;11(2):183-188.

59 Hermann M, Bogunovic D. ISG15: in sickness and in health. *Trends Immunol* 2017;38(2):79-93.

60 Liu XW, Gao N, Dong C, Zhou L, Mi QS, Standiford TJ, Yu FS. Flagellin-induced expression of CXCL10 mediates direct fungal killing and recruitment of NK cells to the cornea in response to Candida albicans infection. *Eur J Immunol* 2014;44(9):2667-2679.

61 Li RD, Wang YX, Ma ZJ, Ma MY, Wang D, Xie GC, Yin YP, Zhang P, Tao KX. Maresin 1 mitigates inflammatory response and protects mice from sepsis. *Mediators Inflamm* 2016;2016:3798465.

62 Serhan CN, Yang R, Martinod K, Kasuga K, Pillai PS, Porter TF, Oh SF, Spite M. Maresins: novel macrophage mediators with potent antiinflammatory and proresolving actions. *J Exp Med* 2009;206(1):15-23.

63 Tang Q, Che CY, Lin J, He H, Zhao WY, Lv L, Zhao GQ. Maresin1 regulates neutrophil recruitment and IL-10 expression in Aspergillus fumigatus keratitis. *Int Immunopharmacol* 2019;69:103-108.

64 Bozza S, Fallarino F, Pitzurra L, Zelante T, Montagnoli C, Bellocchio S, Mosci P, Vacca C, Puccetti P, Romani L. A crucial role for tryptophan catabolism at the host/Candida albicans interface. *J Immunol* 2005;174(5):2910-2918.

65 Jiang N, Zhao GQ, Lin J, Hu LT, Che CY, Li C, Wang Q, Xu Q, Zhang J, Peng XD. Expression of indoleamine 2, 3-dioxygenase in a murine model of Aspergillus fumigatus keratitis. *Int J Ophthalmol* 2016;9(4):491-496.

66 Jiang N, Zhao GQ, Lin J, Hu LT, Che CY, Li C, Wang Q, Xu Q, Peng XD. Indoleamine 2, 3-dioxygenase is involved in the inflammation response of corneal epithelial cells to aspergillus fumigatus infections. *PLoS One* 2015;10(9):e0137423.