CLEC-1 Acts as a Negative Regulator of Dectin-1 Induced Host Inflammatory Response Signature in *Aspergillus fumigatus* Keratitis

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Purpose. C-type lectin-like receptor-1 (CLEC-1) is a member of the Dectin-1 cluster of pattern recognition receptors (PRRs). It is involved in host immunity, has immunoregulatory function, and supports allograft tolerance. Our study aimed to describe the role of CLEC-1 in response to fungal keratitis, in situ, in vivo, and in vitro.

Methods. Quantitative polymerase chain reaction (qRT-PCR) and immunofluorescence were used to detect the expression of CLEC-1 in corneas of patients with *Aspergillus fumigatus* (*A. fumigatus*) keratitis. In vitro and in vivo experiments were designed in THP-1 macrophages and C57BL/6 mouse models, respectively. The expression of CLEC-1 in corneas of mice model was determined by qRT-PCR, Western blot, and immunofluorescence. CLEC-1 overexpression in mouse corneas was achieved by intrastromal injection of adeno-associated virus (AAV) vectors. Disease response was evaluated by slit-lamp photography, clinical score, and colony forming unit (CFU). Bioluminescence imaging system image acquisition, myeloperoxidase (MPO) assays, immunofluorescence staining, qRT-PCR, and Western blot were used to investigate the role of CLEC-1. To further define the role of CLEC-1, we used lentivirus vectors to overexpress CLEC-1 or/and Dectin-1 in THP-1 macrophages.

Results. The expression of CLEC-1 was increased in corneas of patients with *A. fumigatus* keratitis. In corneas of mice from the *A. fumigatus* keratitis model, the expression of CLEC-1 was decreased in the acute inflammatory stage and increased during convalescence. Following Natamycin treatment, CLEC-1 was upregulated in *A. fumigatus* keratitis mice. Compared with normal C57BL/6 mice, overexpression of CLEC-1 converted the characteristic susceptible response to resistance, as demonstrated by slit-lamp photography and clinical score. In vivo studies revealed decreased MPO levels and neutrophils recruitment and higher fungal load after the upregulation of CLEC-1. Compared with control corneas, CLEC-1 overexpression impaired corneal pro-inflammatory cytokine IL-1β production.

Conclusions. These findings demonstrate that CLEC-1 may act as a negative regulator of Dectin-1 induced host inflammatory response via suppressing neutrophils recruitment and production of pro-inflammatory cytokine IL-1β production in response to *A. fumigatus* keratitis.

Keywords: aspergillus fumigatus, keratitis, C-type lectin-like receptor-1 (CLEC-1), Dectin-1, IL-1β

Fungal keratitis is an infectious disease that, when severe, can lead to blindness.[1,2] In developing countries, including Mexico, India, and China, fungal keratitis accounts for 35% of all corneal ulcers, causing severe visual impairment and blindness.[3–5] Even in tropical regions of developed countries, such as in the southeastern United States, fungal keratitis accounts for up to 35%.[6] The predominant risk factor of fungal keratitis is vegetative trauma.[7,8] Understanding the host's immune response to fungal infection is essential to prevent and treat fungal keratitis and other fungal diseases.[9]

The Dectin-1 cluster, part of the pattern recognition receptors (PRRs) in host immunity, includes Dectin-1, LOX-1, CLEC-1, CLEC-2, CLEC-9A, CLEC-12A, and CLEC-12B. This C-type lectin-like receptor (CTLR) family is encoded in the centromeric part of the natural killer (NK) gene complex of human chromosome 12. The CTLR family recognizes a variety of ligands and performs various other immune and homologous functions.[10–12] Dectin-1 is a CTLR that binds endogenous ligands on T cells, mycobacteria, and β-1, 3-glucan. Being a model receptor of CTLRs signal transduction, Dectin-1 is well-studied, and many of its

characteristics are clearly defined. Dectin-1 recognizes β-glucan can result in the induction of a lot of cellular responses. For example, it can give rise to the expression of numerous cytokines and chemokines, including IL-1β, IL-6, IL-10, IL-23, TNF-α, and CXCL2.[13–15] Besides, it also could cause ligand uptake, DC maturation, and respiratory burst. LOX-1, another well-known member of the Dectin-1 cluster, can recognize various ligands, such as bacteria, OxLDL, aged and apoptotic cells, activated platelets, and HSP70. Recognition of these ligands by LOX-1 leads to endocytosis, phagocytosis, antigen cross-presentation, and cytokine production.[16–18]

Compared with LOX-1 and Dectin-1, the function of C-type lectin-like receptor-1 (CLEC-1) is still poorly understood. CLEC-1 is a group V type II transmembrane receptor. Other subfamily members highly homologous to CLEC-1 are CLEC-2, LOX-1, and Dectin-1. The expression of CLEC-1 has been detected in peripheral blood mononuclear cells, endothelial cells, and human dendritic cells.[19] Alloantigen-induced CD4$^+$ CD25$^+$ T cells increased the expression of CLEC-1 in endothelial cells of allografts with tolerance, but not in allografts with rejection. This upregulation is related to the decrease of IL-17 and the increase of Foxp3 expression, demonstrating that CLEC-1 may support allograft tolerance.[20] Furthermore, another report suggests that TGF-β upregulated CLEC-1, whereas inflammatory stimuli or other receptor ligands had little effect on it.[21] Despite these recent findings on the role CLEC-1, its role in fungal keratitis remains unclear.

Our study investigated the role of CLEC-1 receptor in the corneas of patients with *Aspergillus fumigatus* (*A. fumigatus*) keratitis, mice corneas, and human THP-1 macrophages infected with *A. fumigatus*. Our findings suggest that CLEC-1 may act as a negative regulator of Dectin-1 via suppressing the recruitment of neutrophils and the production of inflammatory cytokines in *A. fumigatus* keratitis.

MATERIALS AND METHODS

Clinical Specimens

Six cases of corneas with *A. fumigatus* keratitis and six healthy corneas were collected for immunofluorescence and quantitative polymerase chain reaction (qRT-PCR). Corneas in the control group were obtained from cadaveric specimens without ocular disease. Central donor corneas were used for keratoplasty and the rest of the peripheral corneal tissues were collected for immunofluorescence and qRT-PCR.

Six patients (6 eyes) with *A. fumigatus* keratitis underwent penetrating keratoplasty and corneas with lesions were collected for immunofluorescence and qRT-PCR. The purpose and methods of the study were explained to patients in detail. After informed consent was obtained, samples were collected. Patients with any form of immunosuppression, topical steroid therapy, or acute/chronic systemic disease were excluded from this research. Specimens were confirmed by fungal culture and morphology.

It was approved by ethics committee of Affiliated Hospital of Qingdao University for using these corneas. This study also followed the principles set out in the Declaration of Helsinki.

Preparing *A. fumigatus*

Sabouraud agar was used to culture *A. fumigatus* strain 3.0772 (China General Microbiological Culture Collection Center) for 3 to 4 days. After scraping fresh conidia from the surface of culture medium, the suspension was adjusted to the final concentration of 5×10^4 conidia/µL in PBS quantified by hematology analyzer.

In Vivo Experiments

All mice were treated according to the Statement of Animal Application in Ophthalmic and Vision Research published by the Association for Research in Vision and Ophthalmology (ARVO). Eight percent chloral hydrate (400 mg/kg) was used to anesthetize 8-week C57BL/6 female mice. Corneal stroma was filled with conidia of *A. fumigatus* (1×10^5 /2 µl) with a 33-gauge Hamilton syringe. Corneal opacity was examined with slit lamp microscope every day after infection. The clinical score was recorded in a previously published scoring system,[22] and the disease severity was classified statistically. Corneas were harvested for Western blot and qRT-PCR at 1/2, 1, 2, 3, 5, 7, 10, and 14-days postinfection, and for immunofluorescence staining eyeballs were removed at 5-day postinfection.

To verify the relationship between CLEC-1 expression and disease severity, mice were randomly divided into two groups: one group was not treated after *A. fumigatus* infection, whereas the other group was treated with natamycin after *A. fumigatus* infection of corneas. The degree of corneal infection was observed, the clinical score was recorded, and the expression of TGF-β and CLEC-1 was detected by qRT-PCR and Western blot on day 3 and day 5.

CLEC-1 corneal overexpression in mice was achieved with intrastromal injection of CLEC-1 adeno-associated virus (AAV) vectors (Genechem, Shanghai, China). We selected one eye from each mouse randomly to receive the intrastromal injection (5 µl) 10^{11} vg of vector. Two weeks later, enhanced green fluorescent protein (EGFP) expression was evaluated using a fluorescence stereomicroscope to ensure approximate distribution of the virus in the corneal stroma. The qRT-PCR and Western blot were used to assess the overexpression of CLEC-1. We then harvested corneas for myeloperoxidase (MPO) assays, immunofluorescence staining, Western blot, and qRT-PCR 1-day after infection.

In Vitro Experiments

THP-1 macrophages were purchased from the China Center (Wuhan, China) and cultured as previously published.[18] Lentivirus vectors of CLEC-1 was pretreated in THP-1 macrophages. THP-1 macrophages were infected with *A. fumigatus* conidia at a multiplicity of infection (MOI) of 1 for 16 hours after 24 hours pretreatment, and then harvested for Western blot and qRT-PCR.

Colony Forming Unit Calculation

Whole eyes were homogenized in 1 mL PBS and the mixer scientz-48 (scientz, Ningbo, China) was set to 33 hz for 4 minutes. After that, continuous logarithmic dilution was performed and plated on Sabouraud glucose agar plate (BDMs, Cockeysville, MD, USA). The number of colony forming units CFUs in fungi was determined by direct counting method after cultured at 37°C for 24 hours.

TABLE. Nucleotide Sequences of Mouse Primers for qRT-PCR

Genes	Primer Sequence (5'-3')
hCLEC-1-F	CGTTGTGTCTGTGAGAGA
hCLEC-1-R	CCTTCGCCTAATGTTTCA
mCLEC-1-F	TGTGAATACTTCTGCCTGGCT
mCLEC-1-R	GAGAGCCCTGTCCAATAAGAGTA
hDectin-1-F	TGGACGAAGATGGATATAC
hDectin-1-R	CAAGCACAGGATTCCTA
mDectin-1-F	GACCCAAGCTACTTCCTC
mDectin-1-R	GCAGCACCTTTGTCATACT
mTGF-β-F	AATTCCTGGCGTTACCTTGG
mTGF-β-R	TCTCCTTGGTTCAGCCACTG
hIL-1-β-F	GCTGATGGCCCTAAACAGATGAA
hIL-1-β-R	TCCATGGCCACAACAACTGAC
mIL-1-β-F	CGCAGCAGCACATCAACAAGAGC
mIL-1-β-R	TGTCCTCATCCTGGAAGGTCCACG
hGADPH-F	GCACCGTCAAGGCTGAGAAC
hGADPH-R	TGGTGAAGACGCCAGTGGA
mβ-actin-F	GATTAC TGCTCTGGCTCCTAGC
mβ-actin-R	GACTCATCGTACTCCTGCTTGC

MPO Assay

Corneas, collected 1-day after infection, were cultured in water bath for minutes in the working solution of MPO Kit (njjcbio, Nanjing, China). After culture, the MPO activity of the tissue was measured at 450 nm OD with a microplate reader.

RNA Isolation and qRT-PCR

The mRNA levels of IL-1β, CLEC-1, Dectin-1, and TGF-β were detected by qRT-PCR from human cornea samples, mice corneal samples, or THP-1 macrophages. The PCR method used has been described previously.[18] The primer pair sequences used is shown in the Table.

Western Blot

The Western blot protocol used was previously described.[18] Blots were stained accordingly with anti-CLEC-1 (1:1000, NBP2-27096; Novus Biologicals, Littleton, CO, USA), anti-Dectin-1 (1:1000, mab1704; R&D Systems, Minneapolis, MN, USA), anti-TGF-β (1:1000, 13394-1-AP; Proteintech, Rosemont, IL, USA) and anti-IL-1β (1:1000 AF-401-NA; R&D, USA). HRP-tagged secondary antibodies were purchased from CST.

Immunofluorescence Staining

OCT compound (Tissue-Tek; Miles, Elkhart, IN, USA) was used to embed mouse corneas and human eyes ($n = 6$ /group/time point). The method of immunofluorescence staining has been described in previous publications.[18] Primary antibodies included rabbit anti-human CLEC-1 antibody (1:100; Novus NBP2-27096), rabbit anti-mouse CLEC-1 antibody (1:100; ProteinTech 13394-1-AP), mouse anti-human Dectin-1 antibody (1:100; Novus mab1859), and rat anti-mouse NIMP-R14 antibody (1:100; Santa Cruz sc-59338), used for their respective organisms. Alexa Fluor 555-conjugated goat anti-rabbit antibody (1:1000; CST), Alexa Fluor 555-conjugated goat anti-mouse antibody (1:1000;

CST), and Alexa Fluor 488-conjugated goat anti-rat antibody (1:1000; CST) were used as secondary antibodies.

Bioluminescence Imaging System Image Acquisition and Analysis

Bioluminescence images were captured by the In Vivo Imaging System (IVIS) spectrum (Perkin Elmer, Santa Clara, CA, USA) and analyzed by IVIS imaging software (Perkin Elmer). Imaging examination was performed 1 day after conidia infection of *A. fumigatus*. Seven minutes before imaging, the luminescent reagent (MPO XenoLight RediJect inflammation Probe, Perkin Elmer) was dropped onto the corneal surface of mice. Animals were placed on the warm phase of IVIS, and the infected eyes were placed in the lateral decubitus position. The ocular surface directly facing the camera sensor. The standard optimization scheme was used to determine the imaging parameters in vivo. Mice were imaged with "C" field of vision and using cultured medium for 1 minute. The total bioluminescence of infected eye was determined as the sum of the background-subtracted ocular region of interest (ROI) flux from 2 consecutive 5-minute imaging windows. Normal eye total bioluminescence was determined as 2 times the background-subtracted ocular ROI flux from 5-minute imaging window. Shapiro Wilk analysis was used to test the normality of different paired data, then paired *t*-test in prism-5 software was used to compare the mean and the peak of bioluminescence.[23,24]

Statistical Analysis

Statistical analysis was performed for qRT-PCR, Western blot, clinical score, and CFU assays results using an unpaired, two-tailed, Student *t*-test. Spearman rank correlation coefficient was used to describe the correlation between CLEC-1 and Dectin-1 in qRT-PCR. A *P* value < 0.05 was considered significant. All experiments were repeated to ensure the reproducibility and representative data were shown as mean ± SD.

RESULTS

CLEC-1 was Involved in Human *A. fumigatus* Keratitis

The qRT-PCR and immunofluorescence staining were used to examine the expression of CLEC-1 and Dectin-1 mRNA and protein in normal human corneas and human corneas of patients with *A. fumigatus* keratitis. The expression of CLEC-1 mRNA was significantly increased in infected corneas compared with healthy corneas. The expression of CLEC-1 mRNA was not significantly changed when the course of the disease was less than 1 month. However, when the course of disease was more than 1 month, the expression of CLEC-1 mRNA was significantly higher (Fig. 1A). The difference was that the expression of Dectin-1 mRNA was increased no matter the course of disease was less than 1 month or more than 1 month, and the expression of Dectin-1 was higher when disease was less than 2 month (Fig. 1D). Meanwhile, compared with normal corneas (Fig. 1B), CLEC-1 protein (red) was observed to be significantly higher (Fig. 1C; A, epithelium; B, stroma; and C, inflammatory cells). Similarly, compared to normal corneas (Fig. 1E), Dectin-1 protein (red) expression also increased when infected with fungal keratitis (Fig. 1F; A, epithelium; B, stroma; and C, endothelium).
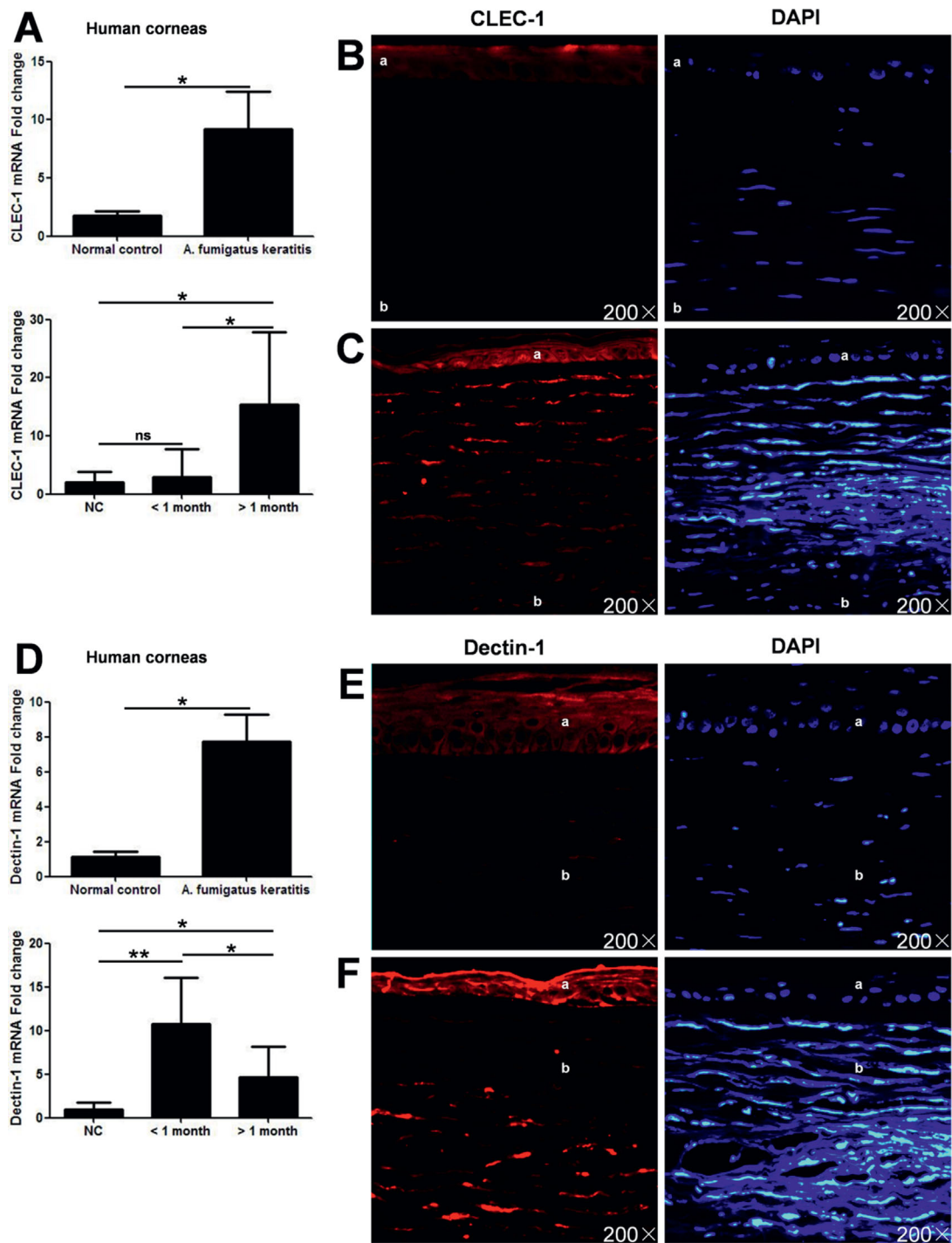
FIGURE 1. CLEC-1 was involved in human *A. fumigatus* keratitis. CLEC-1 mRNA expression (**A**) and Dectin-1 mRNA expression (**D**) in corneas (6 per group) of patients with *A. fumigatus* and healthy donors as determined by qRT-PCR. According to the course of the disease, the analysis was divided into less than 1 month and more than 1 month. The expression of CLEC-1 protein in the corneas of healthy donors (**B**) and patients with *A. fumigatus* keratitis (**C**) was observed by immunofluorescence staining with 7 μm frozen sections. Red represents CLEC-1, blue represents DAPI. Similarly, Dectin-1 protein expression in the corneas of healthy donors (**E**) and patients with *A. fumigatus* keratitis (**F**) was observed by immunofluorescence staining. Red represents Dectin-1, blue represents DAPI (**A**, epithelium; **B**, stroma; and **C**, endothelium). Magnification × 200. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, ns = nonsignificant.

The Expression of CLEC-1 in Mice *A. fumigatus* Keratitis

Significant corneal opacity (Fig. 2A) and clinical scores (Fig. 2B) were observed in C57BL/6 mice 1 day after infec-

tion and lasted until 14-days postinfection. With the increase of neovascularization, keratitis was gradually improved. Compared with the control group, the qRT-PCR results showed that the level of CLEC-1 protein in the cornea of infected mice decreased at 12 hours and persisted up to
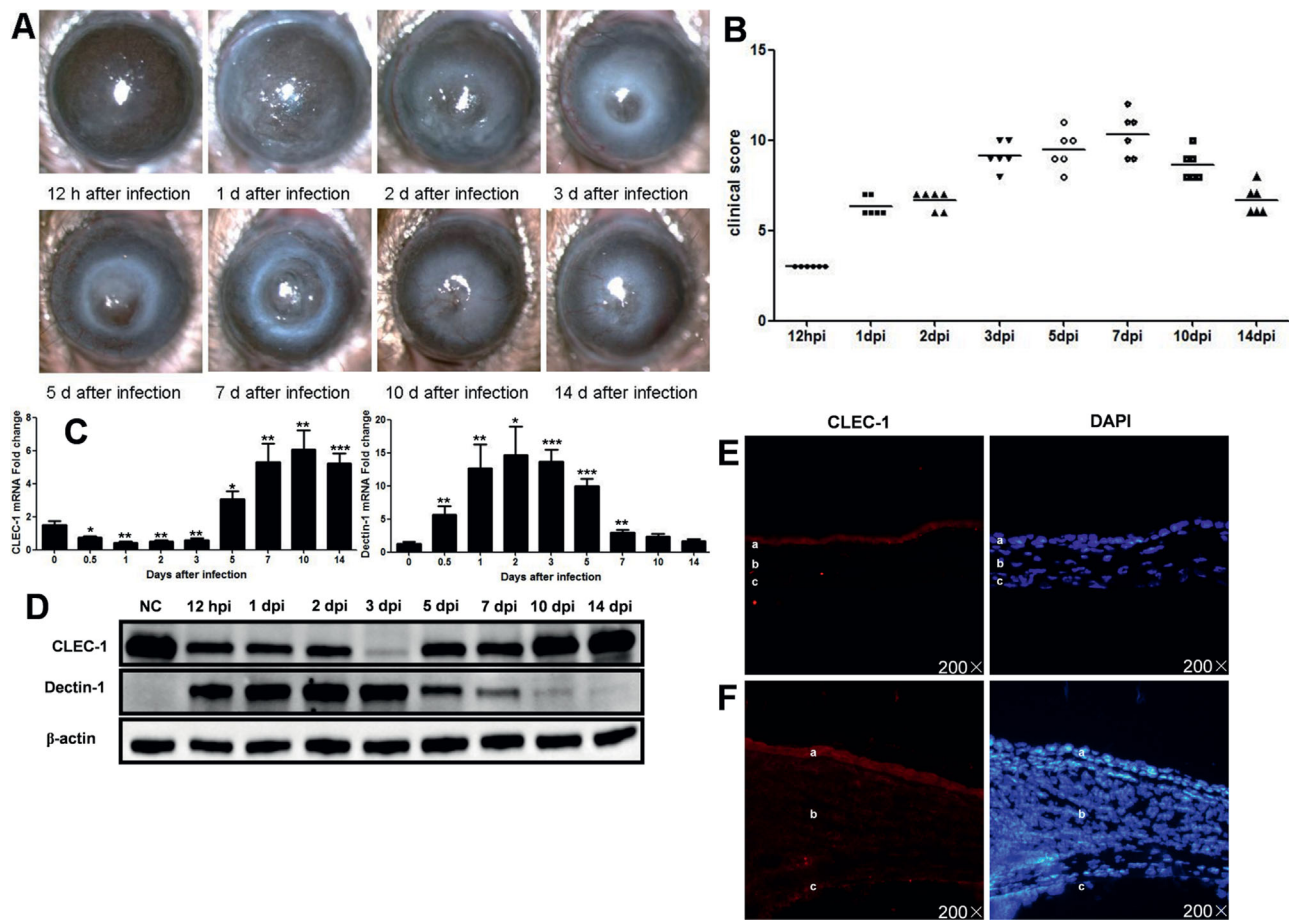
FIGURE 2. CLEC-1 expression of in a mouse model of *A. fumigatus* keratitis. Typical corneal photos of *A. fumigatus* keratitis model in mice were prepared by intrastromal injection (1×10^5 conidia/cornea) at 0, 1 / 2, 1, 2, 3, 5, 7, 10, and 14 days after infection (**A**). Clinical scores were recorded (**B**). Corneas were excised from euthanized mice (6 in each group) at the corresponding time, and qRT-PCR (**C**) and Western blot (**D**) were performed to detect the expression of CLEC-1 and Dectin-1. The expression of CLEC-1 protein in the cornea of the control group and *A. fumigatus* keratitis mice models was observed by immunofluorescence staining (**E, F**) with 7 μm frozen sections. Red represents CLEC-1, and blue represents DAPI (**A**, epithelium; **B**, stroma; and **C**, endothelium). Magnification × 200.

3 days postinfection. From the fifth day, the expression of CLEC-1 increased significantly and persisted up to 14 days after infection. As for Dectin-1, we observed elevated protein levels in *A. fumigatus* keratitis mice models at 12 hours and up to 7 days postinfection, and there was a negative correlation between the expression of CLEC-1 and Dectin-1(rs = −0.567, $P < 0.001$; Fig. 2C). The expressions of CLEC-1 and Dectin-1 were also confirmed by Western blot (Fig. 2D). Immunofluorescence staining also showed that CLEC-1 protein (red) expression increased in infected mice corneas 5-day after infection (Fig. 2F) as compared to controls (Fig. 2E; A, epithelium; B, stroma; and C, endothelium).

CLEC-1 was Upregulated in Natamycin Treated *A. fumigatus* Keratitis Mice

Mice were divided into two groups randomly: one group was infected with *A. fumigatus* keratitis and not treated, whereas the other group was treated with Natamycin after *A. fumigatus* infection. Photographs were taken by slit-lamp 1 day, 3 days, and 5 days postinfection (Fig. 3A). The mice corneas'

clinical scores in the Natamycin-treated group were significantly lower than control group both on day 1, day 3, and day 5 (Fig. 3B). The qRT-PCR result (Fig. 3C) and Western blot result (Fig. 3D) demonstrated that the expression of TGF-β and CLEC-1 was upregulated treated with Natamycin when infected with *A. fumigatus* infection in mice.

Disease Response After CLEC-1 Overexpression Treatment

Figure 4A shows the expression of EGFP in mouse cornea, confirming upregulation of CLEC-1. The qRT-PCR (Fig. 4B) and Western blot results (Fig. 4C) confirmed CLEC-1 overexpression in the corneas of mice *A. fumigatus* keratitis models. Photographs were taken by slit lamp 1 day postinfection (Fig. 4D) and the clinical scores (Fig. 4E) illustrated the effect of CLEC-1 on diseases, such as *A. fumigatus* keratitis in mice infection models. Compared with the control group, the clinical score and the CFU count (Fig. 4F) were decreased after CLEC-1 overexpression. The average bioluminescence of the inflammatory probe on the corneal surface demonstrated that the level of MPO in the
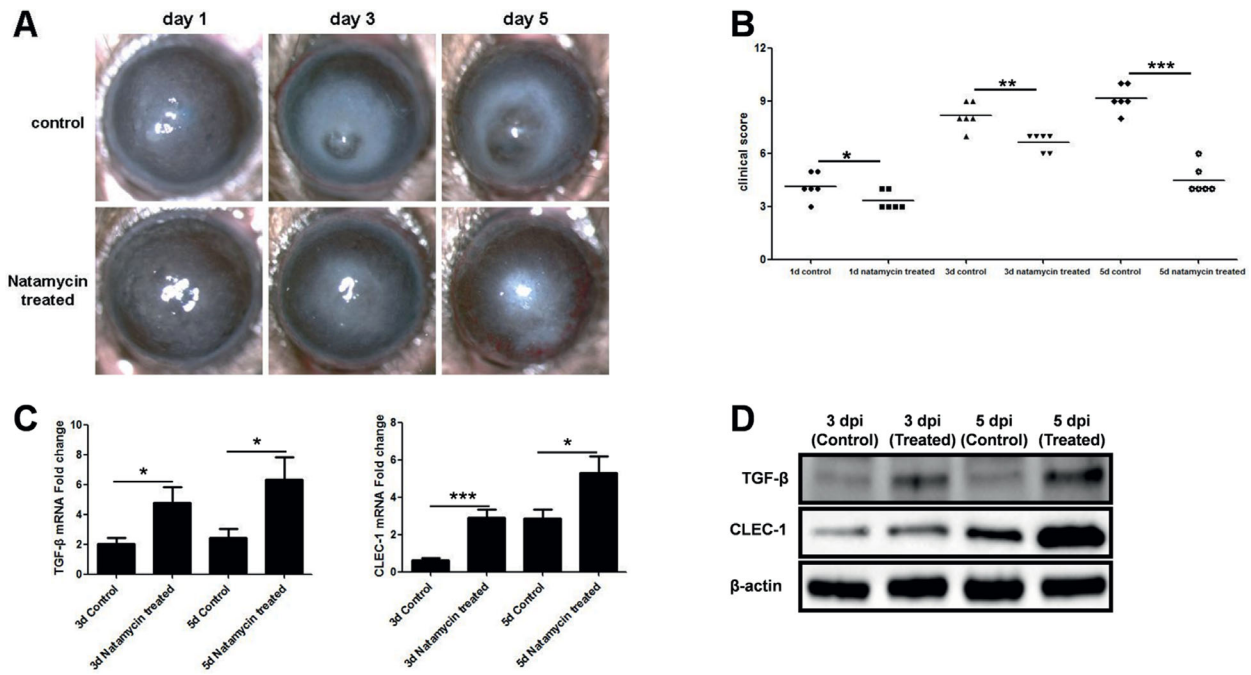
FIGURE 3. CLEC-1 is upregulated in Natamycin-treated *A. fumigatus* keratitis mice. Typical corneal photos of *A. fumigatus* keratitis mice models and Natamycin treated mice models after *A. fumigatus* keratitis at 1, 3, and 5 days after infection (**A**). Clinical scores were recorded on days 1, 3, and day 5 postinfection (**B**). The qRT-PCR (**C**) and Western blot (**D**) were performed to detect the expression of TGF-β and CLEC-1.
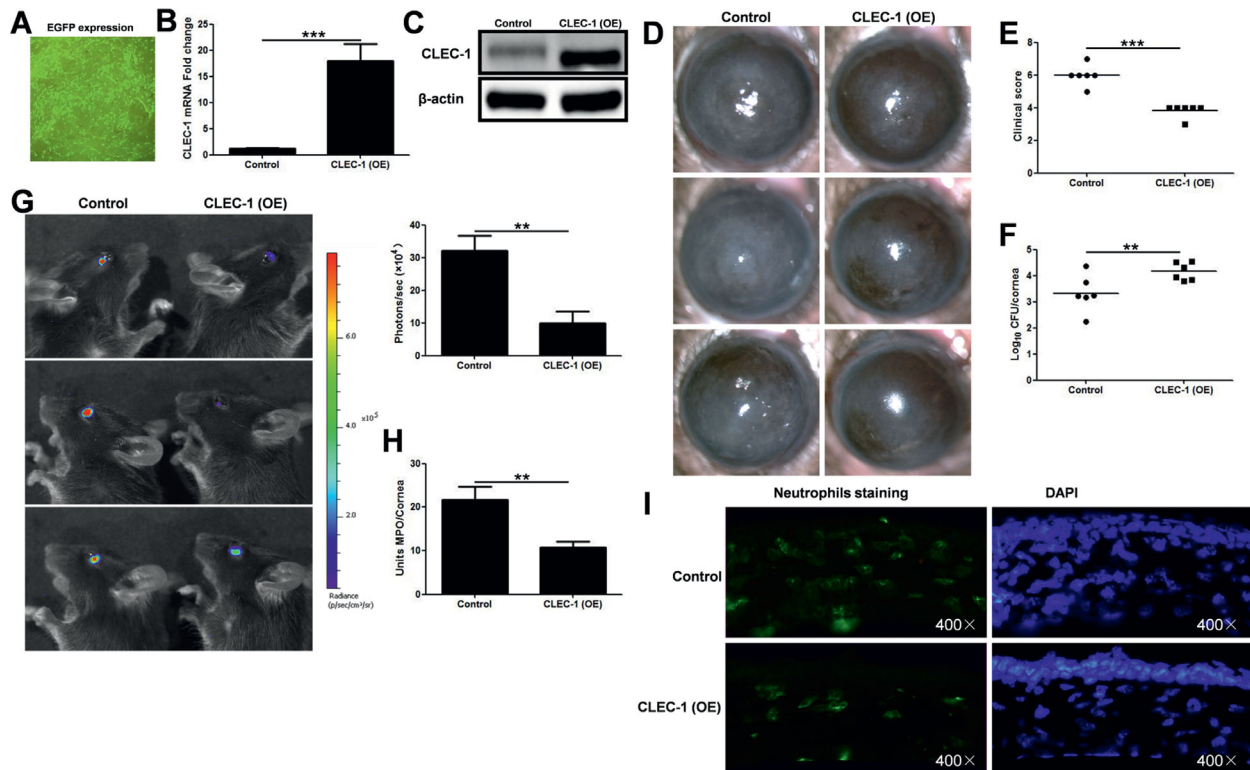

FIGURE 4. Disease response after CLEC-1 overexpression treatment. The expression of EGFP was detected by fluorescence microscope 2 days after AAV injection (**A**). Effective overexpressing of CLEC-1 was confirmed with mRNA (**B**) and protein levels (**C**). Representative corneal images of *A. fumigatus* keratitis mice models 1 day postinfection with or without CLEC-1 overexpression (**D**), and clinical scores were recorded (**E**). CFU calculation was used to detect the fungal load on mice corneas one day post-infection (**F**). The MPO level of the surface of *A. fumigatus* infected mice corneas was measured by the average bioluminescence. Representative images and photon detection are presented (**G**). The corneas of mice euthanized 1 day postinfection were excised and subjected to a colorimetric activity assay for MPO (**H**). The 7 μm frozen sections were immunostained to visualize neutrophil infiltration (*green*) in *A. fumigatus* infected mice corneas with or without CLEC-1 overexpression (**I**). Magnification × 400.
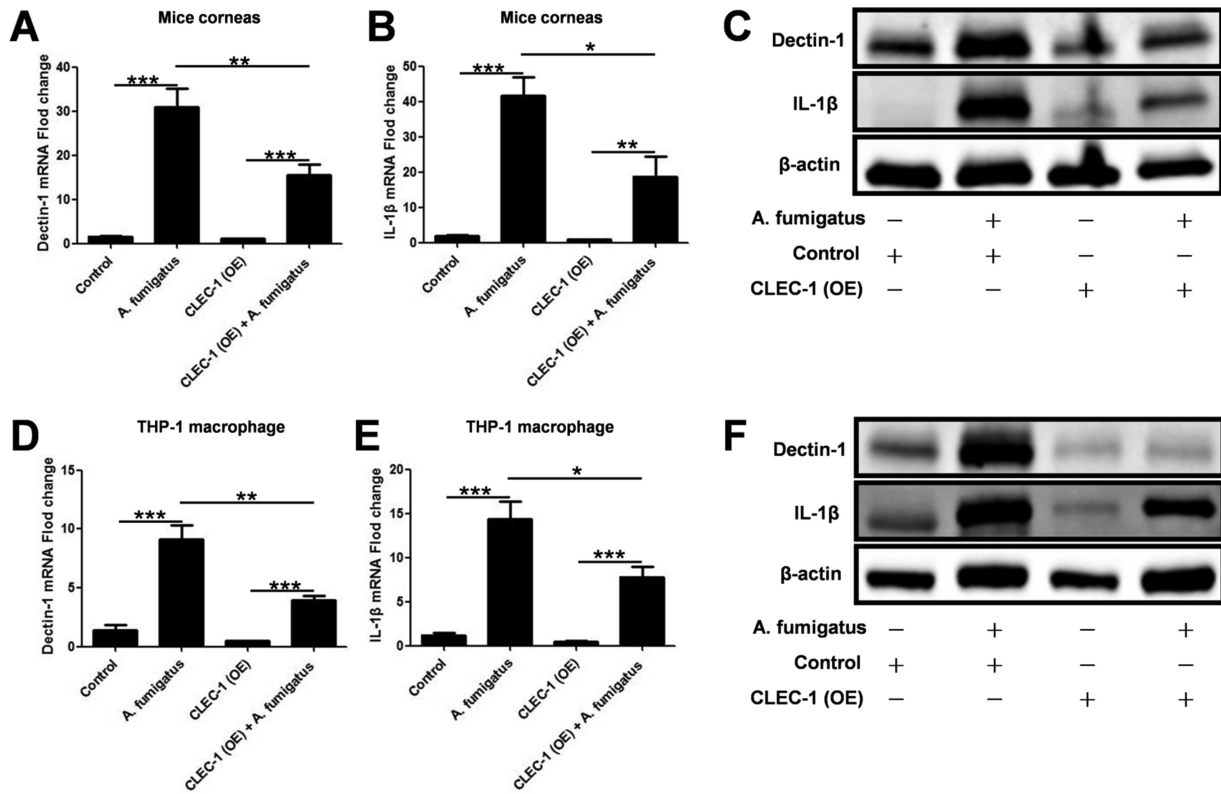
FIGURE 5. CLEC-1 overexpression suppresses Dectin-1 and IL-1β expression in response to *A. fumigatus* infection. The mice were randomly divided into four groups: control group, *A. fumigatus* infection group, CLEC-1 overexpression group, and CLEC-1 over-expression with *A. fumigatus* infection group. The qRT-PCR (**A, B**) and Western blot (**C**) were performed to detect the expression of CLEC-1 and IL-1β. THP-1 macrophages were stimulated with *A. fumigatus* conidia (MOI = 1) for 16 hours after CLEC-1 overexpression. Cells were then subjected to RT-PCR (**D, E**) and Western blot (**F**) for CLEC-1 and IL-1β.
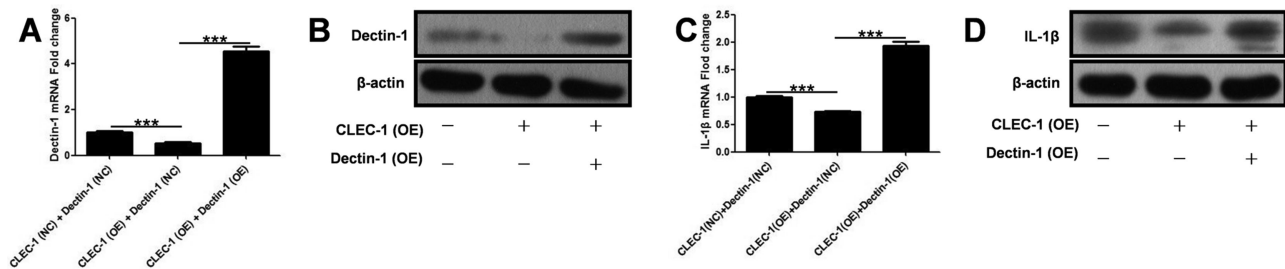

FIGURE 6. CLEC-1 acts as a negative regulator of Dectin-1. After CLEC-1 over-expression or both CLEC-1 and Dectin-1 overexpression, qRT-PCR (**A, C**) and Western blot (**B, D**) were performed to detect the level of CLEC-1 and IL-1β in THP-1 macrophages.

CLEC-1 overexpression group decreased significantly 1 day postinfection (Figs. 4G, 4H). Corneal staining (green) of CLEC-1 overexpression mice models indicated a decrease in neutrophils compared with the control group (Fig. 4I).

CLEC-1 Overexpression Suppressed Dectin-1 and IL-1β Expression in Response to *A. fumigatus* Infection

The role of CLEC-1 upon *A. fumigatus* infection was confirmed by in vivo and in vitro experiments. After the CLEC-1 overexpression, we observed Dectin-1 mRNA (Figs. 5A, 5D) and protein (Figs. 5C, 5F) expression levels significantly lower in corneas of mice models THP-1 macrophages. Similarly, the expression of IL-1β mRNA (Figs. 5B, 5E) and

protein (Figs. 5C, 5F) upon *A. fumigatus* infection was also downregulated in corneas of mice models and THP-1 macrophages.

CLEC-1 Acted as a Negative Regulator of Dectin-1

To confirm the relationship between CLEC-1 and Dectin-1, we overexpressed CLEC-1 alone, or combined with Dectin-1 overexpression, then detected the expression of Dectin-1 and IL-1β using qRT-PCR and Western blot. The expression of Dectin-1 decreased after CLEC-1 overexpression. In contrast, the expression of Dectin-1 increased when CLEC-1 and Dectin-1 were overexpressed (Figs. 6A, 6B). As for IL-1β, the expression decreased after CLEC-1 overexpression, whereas the expression was rescued with CLEC-1 and Dectin-1 overexpressed (Figs. 6C, 6D).

DISCUSSION

CLEC-1 is a member of the C-type lectin-like receptor (CLR) subfamily, encoded in the human natural killer gene complex. CLR consists of several pattern recognition receptors that are important for innate immune function. Although CLRs are known to play a critical role in antifungal immunity,[25] little is known on the function of CLEC-1. CLEC-1 can activate downstream Th17 responses and inhibit dendritic cell activation.[26] Moreover, inflammatory stimulation down-regulated the expression of CLEC-1, and immunomodulatory mediators upregulated the expression of CLEC-1.[20] Most of the current studies were related to immune escape function of CLEC-1. Therefore, the role of CLEC-1 in fungal keratitis is still not fully understood.

Our study found that the expression of CLEC-1 did not increase in the human cornea when the course of *A. fumigatus* keratitis was less than 1 month but increased significantly when the course of the disease was longer. However, in the mouse cornea, the expression of CLEC-1 decreased at first and then increased gradually, which was different from human beings. The difference of CLEC-1 expression may be related to the different fungal keratitis progression between human and mouse corneas. Human fungal keratitis has a long course and is not easy to be cured, whereas mouse fungal keratitis has a short disease cycle, with the symptoms gradually worsen at the beginning of the course and gradually alleviate. In our study, after the application of Natamycin, the symptoms of *A. fumigatus* keratitis were reduced, and the expression of CLEC-1 was increased. This suggested that CLEC-1 was associated with the course of disease and severity of symptoms. CLEC-1 could recognize Melanin (another name of CLEC-1) in conidial spores of *A. fumigatus* and other DHN-melanized fungi in mice to protect against disseminated infection with *A. fumigatus*.[27] Therefore, the expression of CLEC-1 decreased in the early stage of fungal keratitis mainly infected by hyphae in fungal keratitis, whereas the expression of CLEC-1 was significantly upregulated after the hyphae were killed. Then cornea restored the recognition of spores by the increased CLEC-1. Consistent with our findings, TGF-β has been shown to induce CLEC-1 overexpression.[21]

Dectin-1, as a β-glucan receptor, is one of the best-characterized receptors in the Dectin-1 cluster and belongs to the C-type lectin superfamily.[28] Dectin-1 has proved to recognize fungal species, such as *A. fumigatus*.[29] Dectin-1's recognition of those organisms triggers many protective responses, such as ingestion of fungi by phagocytosis and killing fungi by respiratory burst. Our previous study has shown that Dectin-1 plays an important role in the production of IL-1β through JNK activation and apoptosis in *A. fumigatus* keratitis.[30] CLEC-1 comprises a subfamily of CLRs with largely myeloid expression and its closest homologs, Dectin-1, LOX-1, and CLEC-2.[19,31] Although CLEC-1 and Dectin-1 belong to the same family, the relationship between CLEC-1 and Dectin-1 is not clear.

In our study, the expression of CLEC-1 and Dectin-1 was increased with *A. fumigatus* in humans. The corneal expression of CLEC-1 was upregulated for over a month, whereas the expression of Dectin-1 was upregulated for less than a month. In corneas of mice *A. fumigatus* keratitis model, the expression of CLEC-1 was decreased in the acute inflammatory stage and increased during convalescence. However, the expression of Dectin-1 has an opposite pattern. The expression pattern of CLEC-1 and Dectin-1 was different because they recognize different substances. Dectin-1 is involved in antifungal immunity by recognizing β-glucan on the surface of fungal cell walls,[9,32,33] but CLEC-1 recognized the spores of *A. fumigatus*, which showed great differences. When CLEC-1 was overexpressed, the expression of Dectin-1 decreased in the cornea of mice and THP-1 cells with *A. fumigatus*. This showed that CLEC-1 could negatively regulate Dectin-1 expression.

Moreover, in *A. fumigatus* keratitis, Dectin-1 and other pattern recognition receptors (PRPPs) can be activated by *A. fumigatus* to promote inflammatory factors and induce neutrophil aggregation. Neutrophils could kill fungi, but they could also cause irreversible tissue damage, leading to decreased corneal transparency. Seen from the slit-lamp photography and clinical score, overexpression of CLEC-1 converted the characteristic susceptible response to resistance compared to the normal course of disease in C57BL/6 mice. In vivo studies showed decreased MPO levels, fewer recruited neutrophils, and higher fungal load after upregulating CLEC-1 expression. CLEC-1 overexpression could impair corneal pro-inflammatory cytokine IL-1β production. CLEC-1 combined with antifungal antibiotics could provide an opportunity to kill pathogenic fungi in the cornea and maintain corneal transparency.

In conclusion, our findings demonstrated that CLEC-1 might act as a negative regulator of Dectin-1 induced host inflammatory response signature via suppressing neutrophils recruitment and pro-inflammatory cytokine IL-1β production in response to *A. fumigatus* keratitis.

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