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ORIGINAL RESEARCH

CXCL1 and CXCL2: Key Regulators of Host Defense Against *Phialophora verrucosa*

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Purpose: Dematiaceous fungi, such as *Phialophora verrucosa* (*P. verrucosa*), cause persistent infections that are difficult to treat. The purpose of this study was to investigate the roles of chemokines CXCL1 and CXCL2 in the innate immune defense against *P. verrucosa* infections.

Methods: A subcutaneous infection model was employed, in which live *P. verrucosa* conidia were administered into the footpads of wild-type C57BL/6 mice and their *Cxcl1* and *Cxcl2* knockout (KO) counterparts (equal numbers of male and female mice). The natural course of infection, pathological changes, and immune responses were monitored continuously over four weeks.

Results: The current results show that both CXCL1 and CXCL2 deficiencies impair fungal clearance, leading to prolonged infection as evidenced by higher fungal loads and histopathology in *Cxcl1*/*Cxcl2* KO mice at week 4. In *Cxcl1* KO mice, increased levels of inflammatory cytokines IFN-γ, G-CSF, and IL-4 were observed, likely compensating for the immunological deficiencies caused by the lack of CXCL1. Conversely, *Cxcl2* KO mice exhibited impaired neutrophil infiltration early in infection, accompanied by a significant increase in macrophage infiltration.

Conclusion: These findings highlight the critical roles of CXCL1 and CXCL2 in mounting an effective immune response against dematiaceous fungi and suggest their potential as therapeutic targets.

Keywords: CXCL1, CXCL2, *Phialophora verrucosa*, immune response

Introduction

Dematiaceous fungi, commonly referred to as "black yeast", are a broad category of harmful pathogenic fungus distinguished by the presence of melanin granules on the fungal cell wall. They are mostly found in soil worldwide. These fungi cause chronic infections that can affect both immunocompromised and immunocompetent individuals, manifesting as chromoblastomycosis, phaeohyphomycosis, mycetoma, or systemic infections.[1–5] In China, *Phialophora verrucosa* (*P. verrucosa*) emerges as a predominant pathogen alongside *Exophiala spinifera* and *Exophiala dermatitidis*.[6,7] Due to their lethality and treatment challenges, dematiaceous fungal infections have garnered increasing attention, yet the pathophysiology and host immune interactions remain poorly understood. Recent studies link caspase recruitment domain-containing protein 9 (CARD9) deficiency with increased susceptibility to these infections, notably through impaired secretion of CXCL1 and CXCL2 chemokines,[8–10] highlighting the critical role of these molecules in host defense against dematiaceous fungi.

The CXCLs-CXCR2 biological axis, formed by the interaction between chemokines CXCL1 and CXCL2 and their receptor CXCR2, plays a pivotal role in intercellular communication and cell migration. This axis activates various signaling pathways that regulate cytokine and chemokine expression, impacting immune responses.[11–13] In *Candida albicans* (*C. albicans*) infection,

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neuroglial cells mediate protective neutrophil recruitment via the CARD9-IL-1β-CXCL1-CXCR2 pathway, facilitating pathogen clearance.[13] Similarly, in *Aspergillus fumigatus* (*A. fumigatus*) infection, significant upregulation of CXCL1 and CXCL2 enhances host immune defense through CXCR2-mediated neutrophil recruitment.[14] Further investigations demonstrate stage-specific regulation of neutrophil trafficking: early-phase MyD88-dependent CXCL1 production followed by CARD9-mediated CXCL2 expression in later stages.[10] This temporally coordinated mechanism ensures precise spatiotemporal control of neutrophil trafficking, facilitating synergistic pathogen clearance.

While the immunomodulatory functions of CXCL1/CXCL2 are well established in common fungal pathogens, their role in *P. verrucosa* infections remains underexplored. Given the rising prevalence of *P. verrucosa* as a primary dematiaceous fungal pathogen in China,[15,16] this study investigates how CXCL1 and CXCL2 modulates host immunity against *P. verrucosa* infections in a mouse model, aiming to provide new insights for therapeutic strategies.

Materials and Methods

Preparation of Conidial Suspension

The *P. verrucosa* (provided by Professor Ruoyu Li from Peking University First Hospital) was selected and transferred to potato dextrose agar (PDA, Solarbio, Beijing, China) slant medium, then incubated in a 28°C incubator for 14–21 days. Two milliliters of phosphate-buffered saline (PBS, Servicebio, Wuhan, Hubei, China) was used to rinse the *P. verrucosa* culture from the PDA slant medium. The resulting suspension was filtered through 8 layers of sterile gauze to remove mycelium and culture medium components. Conidial suspension was washed with PBS three times, centrifuged at 1000 × g for 15 minutes each time, then resuspended in sterile saline to adjust the conidia concentration to 5×10^8 /mL.

Construction of Cutaneous Dematiaceous Fungal Infection Model in Wild-Type and Gene Knockout Mice

Animal experiments were performed on C57BL/6 WT mice (Shanghai Yishang Biotechnology Co., Ltd, Shanghai, China), and *Cxcl1* KO mice (Cat. NO. NM-KO-2111961), *Cxcl2* KO mice (Cat. NO. NM-KO-205466), which were purchased from Shanghai Model Organisms Center, Inc. (Shanghai, China). To minimize inter-individual variability, all mice were on a C57BL/6 background, aged 6–8 weeks and of specific-pathogen-free grade. Each group consisted of 25 mice, with an equal number of males and females (12 males and 13 females). Groups of WT, *Cxcl1* KO or *Cxcl2* KO mice were injected subcutaneously with 100 μL of 5×10^8 /mL live *P. verrucosa* conidial suspension into two hind footpads. They were housed under barrier conditions at Yishang Biotechnology Co., Ltd. in Shanghai.

Observation of General Condition and Lesion in Mice

The general condition, local lesion morphology, ulcers, and limb necrosis of the mice within 4 weeks post-infection were continuously observed and took photos for record-keeping.

Observation of Survival Rate

The mortality of C57BL/6 WT mice and *Cxcl1, Cxcl2* KO mice within 4 weeks post-infection were continuously observed. Each group will retain 5 mice to observe the survival rate.

Measurement of Foot Swelling Rate

The thickness of the hind paw pads of mice (n=5) was measured before and after inoculation on a weekly basis using a vernier caliper to assess the foot swelling rate. Foot Swelling Rate (%) = [(Post-inoculation foot pad thickness (mm) - Pre-inoculation foot pad thickness (mm))/Pre-inoculation foot pad thickness (mm)] × 100%.

Determination of Fungal Load in Foot Lesion Tissue

Under sterile conditions, foot lesion tissues (n=3) were excised, weighed, and transferred to a tissue grinder. Each sample was homogenized in 1 mL of pre-cooled PBS on ice to prepare a tissue homogenate. The homogenates were then serially diluted in sterile PBS at ratios of 1:10, 1:100, and 1:1000. A 100 μL aliquot from each dilution was spread evenly onto

PDA agar plates in duplicate. Following the incubation at 28°C for one week, colony-forming units (CFUs) were counted, and the fungal load was calculated as Log_{10}CFU/g of tissue.

Histopathological Observation

Under sterile conditions, foot lesions were collected and fixed in 4% formalin (Servicebio, Wuhan, Hubei, China) for 24 hours, followed by preparation into paraffin sections. The sections underwent Hematoxylin-Eosin (HE) and Periodic Acid-Schiff (PAS) staining to evaluate histopathological features and fungal morphology.

Assessment of Macrophage and Neutrophil Infiltration in Foot Lesion Tissue

Foot lesions were collected at appropriate time points using sterile scissors. Routine paraffin embedding and sectioning were performed. Immunohistochemical staining for the macrophage marker CD68 and neutrophil myeloperoxidase (MPO) was conducted on the tissue sections. Images were captured under a microscope, and the percentage of positive staining area (%AREA) was calculated using ImageJ software.

Determination of Cytokine Expression in Foot Lesion Tissue Homogenate

Under sterile conditions, foot lesions were excised, weighed, and transferred to a tissue grinder. An appropriate amount of pre-cooled PBS was added to homogenize the tissue on ice, preparing a 10% tissue homogenate. This was then centrifuged at 600×g for 15 minutes at 4°C. Following the instructions provided with the RayPlex MagPro Mouse Inflammation Bead Array 1 (RayBiotech, USA), protein standards were prepared. The samples were then analyzed on a flow cytometer for 12 inflammatory cytokines: G-CSF, IFN-gamma (IFN-γ), IL-10, IL-12 p70, IL-17A, IL-1 beta (IL-1β), IL-2, IL-23 p19, IL-4, IL-6, MCP-1, and TNF-alpha (TNF-α).

The experimental workflow is summarized in Figure 1.

Statistical Analysis

Statistical analysis and graphing were performed using GraphPad Prism 9 software. Data are presented as mean ± SD. Pairwise comparisons between genotypes (WT vs *Cxcl1* KO, WT vs *Cxcl2* KO) at each infection time point were compared using a *t*-test and with $P < 0.05$ considered statistically significant.

Results

Establishment of Animal Models for Subcutaneous Infections by *P. verrucosa*

Subcutaneous injections of 100 μL conidia suspensions of 5×10^8 /mL of *P. verrucosa* were administered to the footpads of mice, as shown in Figure 2A. The appearance of skin lesions and footpad swelling were observed continuously for 4 weeks. The results showed that one-week post-infection, noticeable swelling and ulceration were observed in the footpads of both wild-type (WT) and knockout (KO) mice. However, the extent of swelling in the infected feet of *Cxcl1* KO and *Cxcl2* KO mice was less pronounced compared to WT mice (Figure 2C). By the second week, these lesions had become more severe, characterized by increased swelling and a broader area of ulceration and crusting. By the fourth week of post-infection, the condition improved in WT, *Cxcl1* KO, and *Cxcl2* KO mice, with reduced ulceration and decreased swelling. Meanwhile, both WT and KO mice survived to the end of the observation period, with a survival rate of 100% (Figure 2B).

Deficiency of CXCL1 and CXCL2 Impairs the Mice's Ability to Clear *P. verrucosa*

Tissue from the foot lesions of infected mice was subjected to histopathological examination, as assessed by Periodic Acid-Schiff (PAS) staining. Both in WT mice and KO mice, fungal conidia structures (Figure 3A, indicated by arrows) were visible 3 days post-infection, with a significant increase in inflammatory cell infiltration. By 1 week, the inflammation had become more pronounced, with widespread cellular infiltration and the appearance of fungal hyphae. At 4 weeks, no conidia were observed in WT mice, whereas persistent fungal conidia components were still visible in the *Cxcl1* KO and *Cxcl2* KO mice.

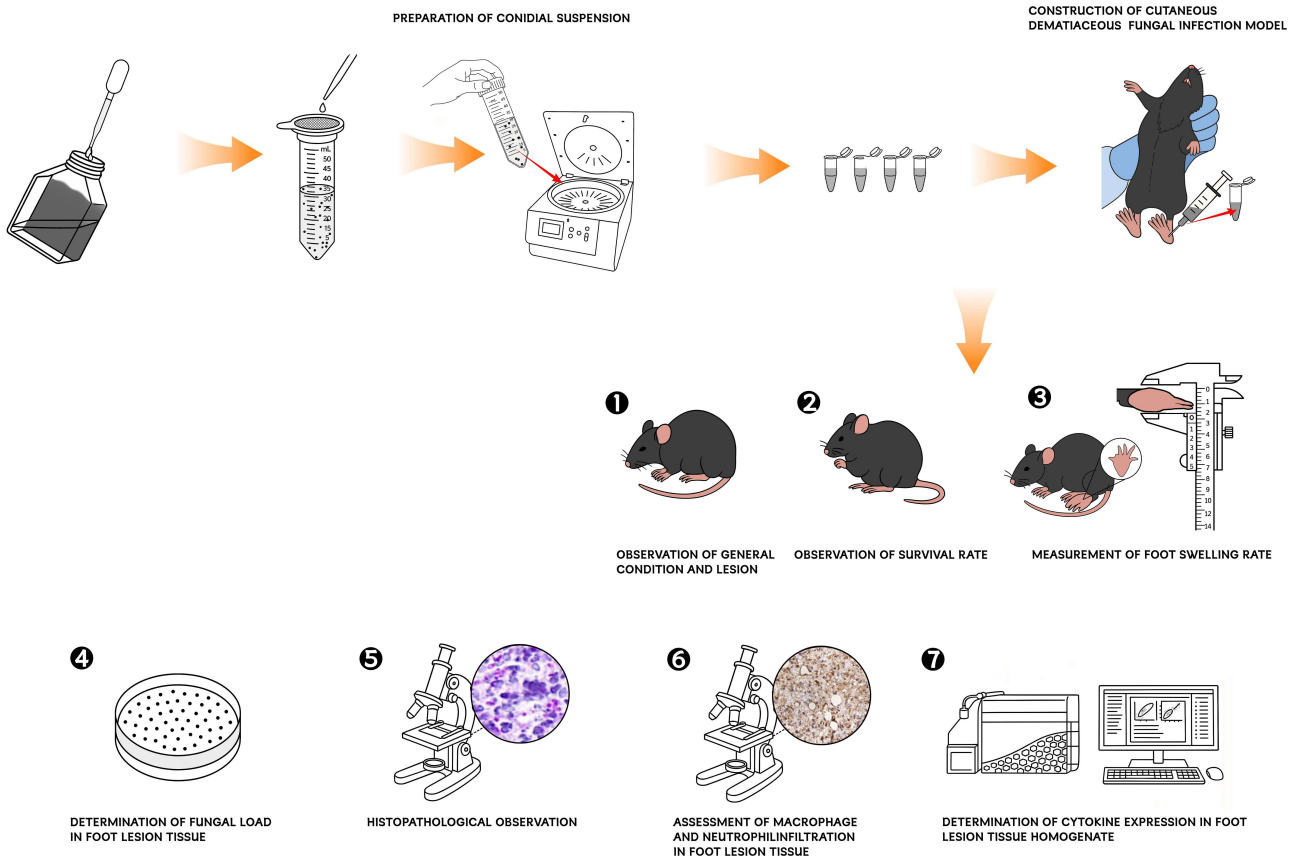

Figure 1 Schematic overview of the experimental design for evaluating CXCL1/CXCL2 roles in *Phialophora verrucosa* infection.

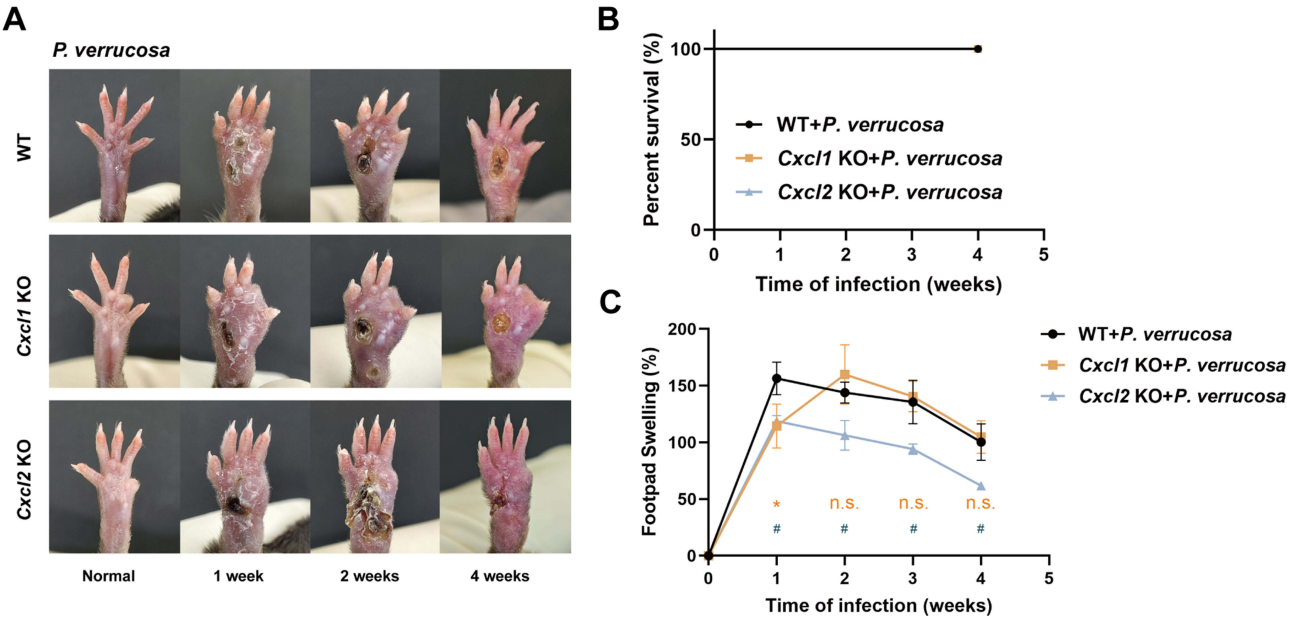

Figure 2 Morphological changes of skin lesions (**A**), survival rate (**B**) and footpad swelling (**C**) in a subcutaneous infection model in mice.
Note: **P*<0.05; #*P*<0.05.

As shown in Figure 3B, in WT mice, colonies of *P. verrucosa* were observed in the tissue one week after inoculation, reaching a peak number of colonies. At the fourth week, no colonies of *P. verrucosa* were detected, indicating that these fungi were completely cleared from the feet of WT mice by the fourth week. In contrast, in *Cxcl1* KO and *Cxcl2* KO mice, the fungal load in the feet reached its peak in the first week, but the infection was cleared slowly. At the fourth week, a significant number of *P. verrucosa* colonies were still present in the lesion tissue, with a statistically significant difference compared to WT mice.

In summary, both the histopathological features and fungal load showed similar trends, indicating that CXCL1 and CXCL2 play crucial roles in the inflammatory response and clearance of *P. verrucosa* in this subcutaneous infection model.

Compensatory Increase in Macrophage Infiltration

The detection of cytokine levels in the skin lesion tissues of mice can reflect the activation status of immune cells. IFN-γ is a key T helper 1 cell (Th1) effector cytokine that can activate macrophages. IFN-γ levels peaked in both WT and KO mice by the first week, with *Cxcl1* KO mice showing significantly higher IFN-γ levels compared to WT mice, which then gradually decreased (Figure 4A). This indicates that CXCL1-deficient mice, due to restricted neutrophil chemotaxis, compensate by releasing large amounts of IFN-γ to activate macrophages and combat *P. verrucosa* infection.

The G-CSF levels in *Cxcl1* KO mice peaked at the second week, significantly higher than those in WT and *Cxcl2* KO mice. By the third week, the G-CSF levels in *Cxcl1* KO mice began to decline but remained higher than in the other groups (Figure 4B). This suggests that CXCL1 deficiency may compensate by stimulating G-CSF secretion through alternative pathways to mitigate the impaired neutrophil chemotaxis during early inflammation.

The levels of the T helper 2 cell (Th2) related effector cytokine IL-4 remained relatively low and similar in both WT and KO mice during the first 2 weeks. By the third week, however, IL-4 levels in *Cxcl1* KO mice significantly increased

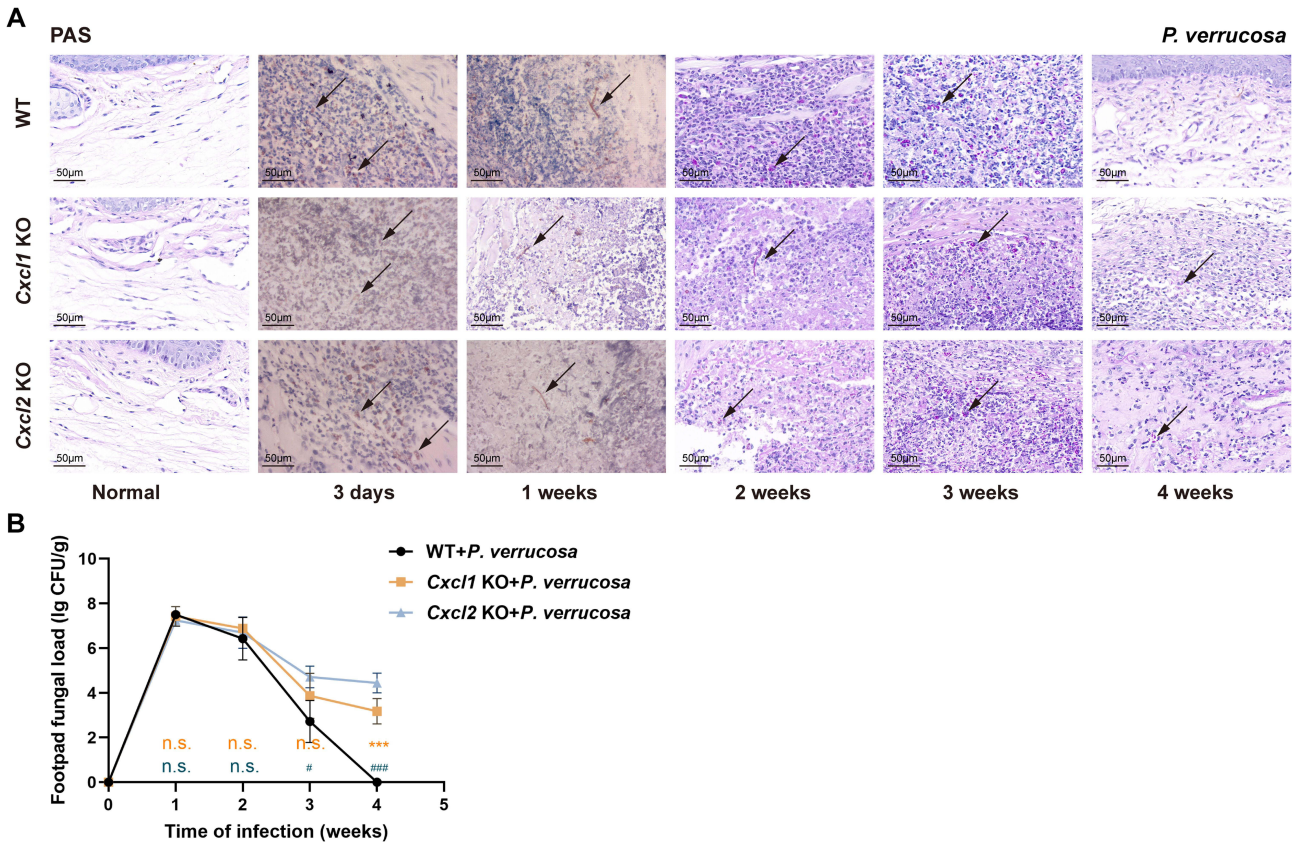

Figure 3 Histopathological features (**A**) and fungal load (**B**) in footpad tissue at different time points following subcutaneous infection with *P. verrucosa* in mice.
Notes: Histopathological analysis of normal footpads and footpad lesions at 3 days and 1, 2, 3, 4 weeks post-infection using PAS staining. Black arrows in (**A**) indicate PAS-positive fungal conidia. $^{\#}P<0.05$; $^{***}P<0.001$; $^{\#\#\#}P<0.001$.

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compared to WT and *Cxcl2* KO mice (Figure 4C), indicating that CXCL1 deficiency promotes Th2 response and activates humoral immunity.

In addition to the significant changes observed in G-CSF, IFN-γ and IL-4 levels, analysis of the remaining nine cytokines (IL-10, IL-12p70, IL-17A, IL-1β, IL-2, IL-23p19, IL-6, MCP-1, and TNF-α) revealed no statistically significant differences between WT and KO groups at all time points (Supplementary Figure S1). To assess the infiltration of macrophages and neutrophils in the early stages of infection, we performed immunohistochemical staining for macrophage markers (CD68) and neutrophil myeloperoxidase (MPO) in skin lesion tissues. We observed and photographed these tissues under a microscope and calculated the percentage of positive area (%AREA). The results showed that in WT mice, macrophage infiltration (CD68%AREA, Figure 5A) remained consistently around 3% over the 14 days of infection, with no significant changes over time. In contrast, neutrophil infiltration (MPO %AREA, Figure 5B) was significantly higher than macrophage infiltration, indicating that neutrophils primarily play an immunological role in WT mice infected with *P. verrucosa*. However, in KO mice, due to the lack of CXCL1 and CXCL2, neutrophil chemotaxis was impaired. By day 7 and day 14, the area of neutrophil infiltration in KO mice was smaller than in WT mice. Notably, by day 14, macrophage infiltration in KO mice was significantly higher than in WT mice, suggesting that the deficiency of CXCL1 and CXCL2 can compensatorily increase macrophage infiltration to combat *P. verrucosa* infection.

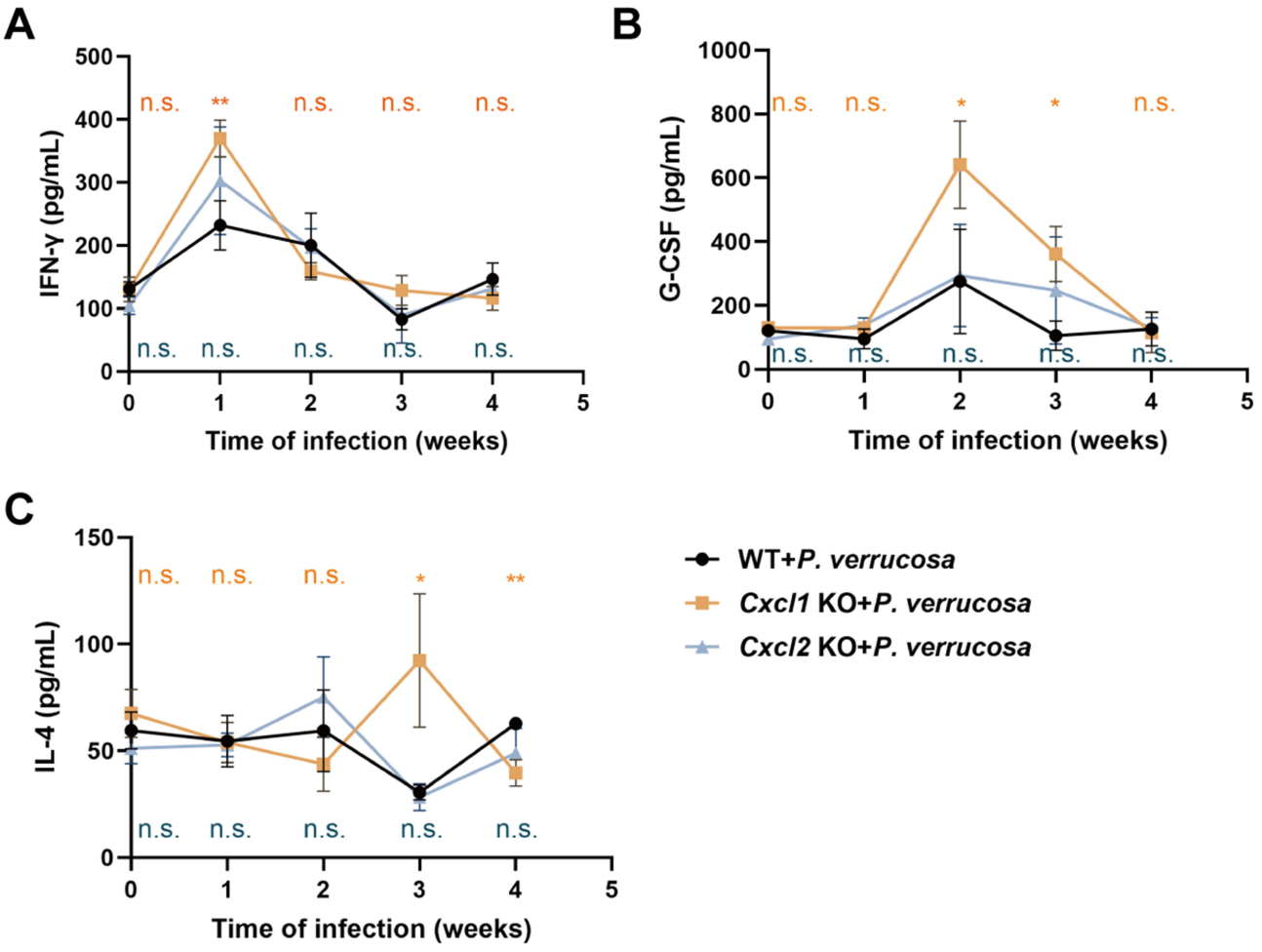

Figure 4 Cytokine expression in tissue homogenates of footpad lesions at different time points in the *P. verrucosa* infection model.
Notes: n=3. (**A**) IFN-γ; (**B**) G-CSF; (**C**) IL-4. *P<0.05; **P<0.01.

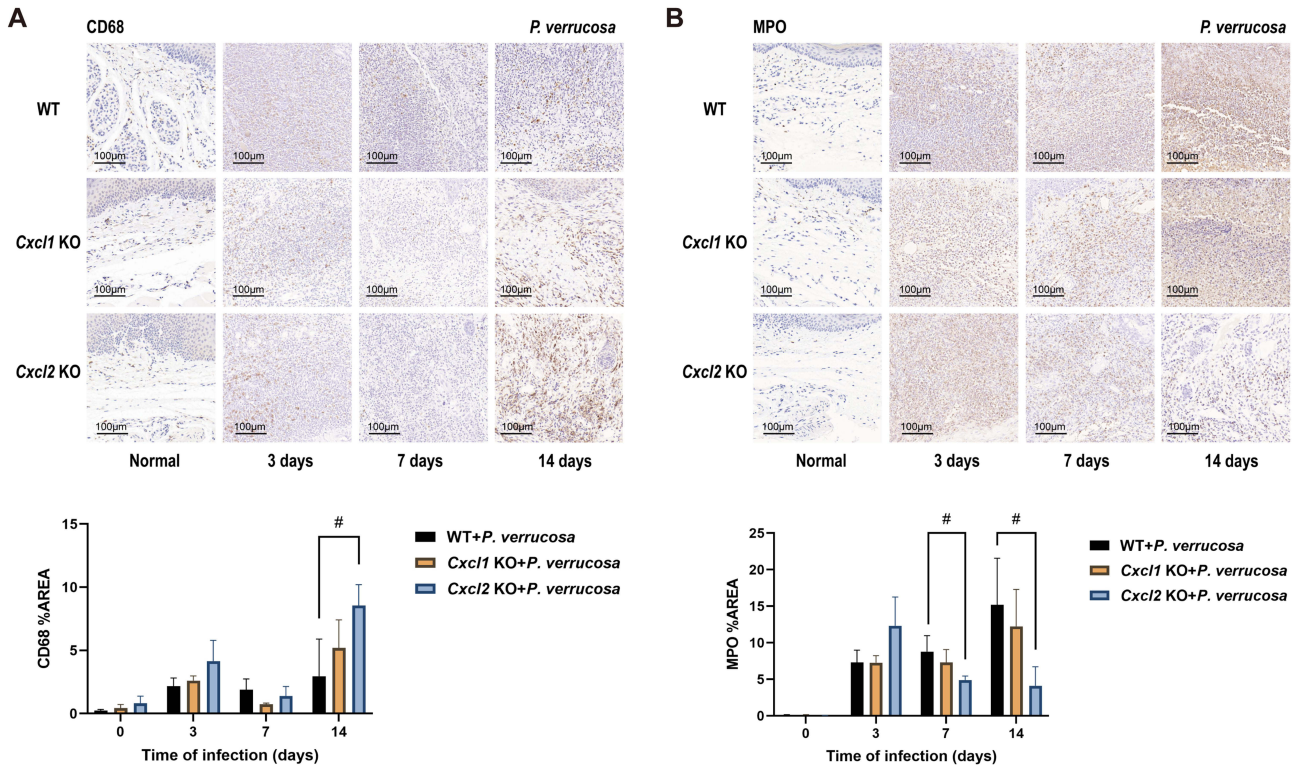

Figure 5 Detection of macrophage and neutrophil infiltration in footpad lesions of mice infected with *P. verrucosa*.
Notes: (**A**) Immunohistochemical staining of macrophage marker CD68 in footpad lesions 14 days post-infection; (**B**) Immunohistochemical staining of myeloperoxidase for neutrophils in footpad lesions 14 days post-infection. $^{\#}P<0.05$.
Abbreviation: %AREA, the percentage of positive staining area.

Discussion

Dematiaceous fungi infections typically invade the human body through skin trauma or breaches, particularly in immunocompromised patients, where the course of illness is often prolonged and difficult to resolve, potentially progressing to systemic infections. Recent studies have revealed that CARD9 plays a critical role in regulating the expression of CXCL1 and CXCL2, and its deficiency leads to insufficient secretion of these chemokines, thereby increasing host susceptibility to dematiaceous fungal infections.[9,10] This underscores the potential importance of CXCL1 and CXCL2 in defending against such infections, with their underlying protective mechanisms warranting further investigation. In this study, we selected *P. verrucosa* as a representative dematiaceous fungus. We established a subcutaneous infection model by injecting live conidia of *P. verrucosa* into the footpads of both hind paws in WT and *Cxcl1*/*Cxcl2* KO mice, mimicking common human infection routes.

In the subcutaneous infection model, both WT and *Cxcl1*/*Cxcl2* KO mice exhibited significant swelling and ulcer formation in the footpad tissue during the first 1–2 weeks post-infection. However, as the inflammatory response progressed naturally, these symptoms gradually subsided, with ulcers crusting over and beginning to heal. Our study observed that both groups of mice eventually showed a tendency toward self-healing, and all mice survived until the end of the experiment. This result is consistent with findings by Wu et al,[17] who used *P. verrucosa* to infect WT mice.

The degree of foot swelling is an important indicator of local infection severity and inflammatory response intensity, reflecting tissue edema and inflammatory cell infiltration. By measuring changes in footpad thickness, we evaluated the extent of foot swelling post-infection. The results showed that foot swelling peaked in the early stages of infection (weeks 1–2) for both WT and *Cxcl1*/*Cxcl2* KO mice, then gradually decreased, indicating that local infection and inflammation progressed from mild to severe and then gradually resolved. Notably, compared to WT mice, the footpad swelling was slightly less pronounced in *Cxcl1*/*Cxcl2* KO mice, particularly in *Cxcl2* KO mice, where swelling remained significantly lower throughout the four-week period. Despite this reduced swelling, *Cxcl2* KO mice exhibited more

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severe ulceration and crusting. We speculate that this may be due to excessive initial swelling leading to tissue rupture and pus discharge, thereby reducing later swelling levels.

To investigate whether deficiencies in CXCL1 and CXCL2 affect the host's ability to clear *P. verrucosa* conidia, we quantified the fungal burden in the infected footpad tissues. Our findings revealed that the rate of fungal clearance was significantly slower in *Cxcl1*/*Cxcl2* KO mice compared to WT mice. By the fourth week post-infection, fungal colonies were still detectable in the *Cxcl1*/*Cxcl2* KO mice. Consistent with these observations, histopathological examinations showed that while WT mice had completely cleared the fungus by week 4, *Cxcl1* and *Cxcl2* KO mice continued to exhibit persistent fungal conidia components. This suggests that the absence of CXCL1 and CXCL2 may delay the clearance of pathogenic fungi and have an adverse impact on the host defense mechanisms.

CXCL1 plays a crucial role in the recruitment of neutrophils to the sites of infection. According to Drummond et al[13], 24 hours after *C. albicans* infection, mice lacking CXCL1 or its receptors CXCR2 showed significantly reduction of neutrophil recruitment compared to WT mice. This deficiency led to more severe fungal infections and poorer pathogen control. Our analysis of cytokine levels in homogenates of infected footpad tissues revealed that G-CSF levels peaked during the second week post-infection in *Cxcl1* KO mice, significantly higher than in WT and *Cxcl2* KO mice. Meng et al[18] demonstrated that G-CSF promotes early granulocyte mitosis and mobilizes cells from the later undivided pool into circulation, which is essential for increasing peripheral blood neutrophil counts and enhancing resistance to fungal infections. These findings suggest that CXCL1 deficiency may stimulate G-CSF secretion through alternative compensatory pathways to compensate for impaired neutrophil chemotaxis during the early inflammatory response. However, the incomplete reversal of fungal clearance defects by G-CSF indicates that quantitative neutrophil recovery alone is insufficient to compensate for functional deficiencies.

We also found that levels of the typical Th1 cytokine IFN-γ peaked during the first week in both WT and *Cxcl1*/*Cxcl2* KO mice, with notably higher levels in *Cxcl1* KO mice compared to WT mice, followed by a gradual decline. This phenomenon may be due to the restricted neutrophil chemotaxis caused by the lack of CXCL1, leading to compensatory increases in IFN-γ production to activate macrophages in response to infection. According to the literature, excessive IFN-γ can have adverse effects on the host. Break et al[19] found that overproduction of IFN-γ by local $CD4^+$ and $CD8^+$ T cells in mice can disrupt the epithelial barrier, increasing susceptibility to *C. albicans* invasion. Wang et al[20] noted that while IFN-γ helps limit the immunopathology of Pneumocystis pneumonia, it also delays fungal clearance. Furthermore, Wang et al[21] revealed that endogenous IFN-γ downregulates macrophage mannose receptor and Dectin-1, thereby weakening the phagocytic activity of alveolar macrophages and promoting the evasion of Pneumocystis from innate immunity. These findings align with our observation that fungal conidia persisted in the lungs of *Cxcl1* and *Cxcl2* KO mice at week 4. Current data suggest that IFN-γ influences the host's ability to combat fungal infections through multiple pathways: on the one hand, it activates macrophages to enhance antigen presentation and killing functions; on the other hand, excessive or persistent IFN-γ may trigger hyperinflammation, exacerbating tissue damage and reducing survival rates. However, the specific mechanisms by which IFN-γ delays the clearance of *P. verrucosa* require further investigation.

Previous studies have shown that in Histoplasma capsulatum infection models using CCR2-deficient ($CCR2^{-/-}$) mice, the absence of CCR2 leads to a significant increase in IL-4 production by alveolar macrophages and dendritic cells.[22] These cells are considered to be alternatively activated, or M2-type macrophages. Notably, IL-4 promotes the survival of intracellular pathogens in M2 macrophages by increasing intracellular zinc ion concentrations through the induction of metallothionein 3 and SLC30A4-dependent pathways.[23] Similarly, our study found that in *Cxcl1* KO mice, IL-4 levels were significantly higher than in WT mice during the third week of post-infection but rapidly declined to below WT levels thereafter. IL-4 is a key cytokine that can induce the polarization of macrophages into the M2 phenotype, which is characterized by functions such as tissue repair, inflammation regulation, and wound healing. Therefore, the elevated IL-4 levels observed during the third week of infection likely contributed to the resolution of inflammation and repair of tissue damage. By the fourth week, as tissues largely healed and returned to normal, IL-4 levels quickly decreased, accelerating the clearance of fungi. This indicates that IL-4 plays a dual role during the infection process: it contributes to pathogen control while also promoting tissue repair and healing. This finding underscores the complexity and importance of IL-4 and its associated signaling pathways in host defense mechanisms.

CXCL1 and CXCL2 play a critical role in promoting neutrophil infiltration. Previous study has shown that during pulmonary *Aspergillus fumigatus* infection, the recruitment of neutrophils occurs in two stages.[10] Stage One: Myeloid

differentiation primary response gene 88 (MyD88) signaling rapidly induces the production of ELR^+ CXC chemokines such as CXCL1 and CXCL5, facilitating the recruitment of neutrophils to the airways. Stage Two: CARD9 regulates the release of CXCL2, which is abundantly produced by the neutrophils themselves, to maintain and amplify subsequent neutrophil recruitment. The study by Zhao et al[24] also demonstrated that during candidiasis, the overproduction of CXCL1 and CXCL2 in the kidneys of Trim26-deficient mice led to excessive neutrophil infiltration. Our study revealed similar temporal dynamics in *P. verrucosa* infection: *Cxcl2* KO mice exhibited significantly reduced MPO positive area percentage in footpad tissues during weeks 1–2 post-infection, confirming CXCL2's essential role in early neutrophil recruitment. Meanwhile, the CD68 positive area percentage (macrophage infiltration) was significantly increased in *Cxcl2* KO mice, suggesting compensatory host defense mechanisms when neutrophil-mediated immunity is impaired. However, emerging evidence from *Fonsecaea monophora* infection models reveals that fungal pathogens may subvert macrophage function through dual mechanisms – promoting M1 polarization while impairing phagocytic capacity (increased CD80 but decreased fungicidal activity), while simultaneously suppressing M2 polarization (reduced CD206) to delay tissue repair.[25] This suggests that the augmented macrophage infiltration in *Cxcl2* KO mice may represent a "double-edged sword": while partially compensating for neutrophil deficiency, aberrant polarization could ultimately compromise fungal clearance. Future studies should characterize macrophage functional phenotypes through M1/M2-specific markers (eg, CD80, CD206). In contrast, the recruitment of neutrophils in *Cxcl1* KO mice was not noticeably impaired, suggesting the presence of redundant mechanisms or alternative pathways that ensure the flexibility of the immune system.

Our study revealed that CXCL1/CXCL2 deficiency may influence host immune responses by modulating the dynamic equilibrium of T helper (Th) cell subsets. During *P. verrucosa* infection, we observed a distinct Th cell polarization shift: *Cxcl1* KO mice exhibited an early Th1-dominant response (elevated IFN-γ), which likely enhanced pathogen clearance but exacerbated inflammatory injury, followed by a late-phase Th2 bias (increased IL-4) that may facilitate immunosuppression and tissue repair.[14,26] Notably, prior studies demonstrated that CARD9 deficiency impairs Th17 function.[17] In line with this, Silva et al[27] reported elevated IL-17 expression but reduced Treg infiltration in lesions of chromoblastomycosis patients, suggesting that this imbalance drives hyperinflammation with inefficient fungal clearance. Our findings posit CXCL1/CXCL2 as pivotal regulators of Th polarization kinetics. Future studies should delineate contributions of other Th subsets (eg, Th17/Th9).

Current evidence remains insufficient to establish CXCL1 or CXCL2 as therapeutic targets for dematiaceous fungal infections such as *P. verrucosa*, where conventional antifungal therapy and surgical intervention remain the primary treatment options.[4] However, emerging immunomodulatory approaches using cytokine antagonists (eg, anti-IL-1 therapy) have shown potential in mitigating excessive inflammatory responses associated with fungal infections,[28] and cytokine therapies including GM-CSF have demonstrated therapeutic efficacy in invasive fungal disease.[29] Our findings regarding the immunopathogenic mechanisms of dematiaceous fungal infections suggest that CXCL1/2-targeted interventions may represent a promising therapeutic strategy, warranting further investigation to elucidate their precise roles in *P. verrucosa* infection and evaluate their potential either as therapeutic targets or prognostic biomarkers, particularly in combination with existing antifungal regimens.

Although our study controlled for known confounders, residual individual variations in immune responses may exist. Importantly, while the murine model has established the critical roles of CXCL1 and CXCL2 in antifungal immunity, future studies should examine the translational relevance of these findings through clinical specimen analysis and evaluate potential therapeutic applications in human-relevant systems for *P. verrucosa* infection management.

Conclusion

In summary, CXCL1 and CXCL2 play crucial roles in the host immune defense against *P. verrucosa* infection. In a subcutaneous footpad infection model, both *Cxcl1* and *Cxcl2* KO mice exhibited delayed clearance of the pathogenic fungus and prolonged disease duration. Specifically, in *Cxcl1* KO mice, increased levels of inflammatory cytokines IFN-γ, G-CSF, and IL-4 were observed, likely as a compensatory response to the immunological deficiencies caused by the lack of CXCL1. Although IFN-γ and G-CSF can enhance macrophage activity and neutrophil numbers, excessive or prolonged expression may affect other immune responses, potentially indirectly promoting the persistence of pathogens. In *Cxcl2* KO mice, impaired neutrophil infiltration during the early stages of infection was noted, accompanied by

a significant increase in macrophage infiltration. These findings highlight the importance of CXCL1 and CXCL2 in orchestrating an effective immune response and suggest potential therapeutic strategies through modulation of these chemokines. Although direct antagonists or agonists targeting CXCL1/CXCL2 require further investigation, emerging immunomodulatory approaches could offer new avenues for managing dematiaceous fungal infections. Future research should focus on understanding the roles of CXCL1 and CXCL2 within the CXCLs-CXCR2 axis and assessing their potential as therapeutic targets or biomarkers in combination with existing antifungal treatments.

Abbreviations

KO, knockout; CARD9, caspase recruitment domain-containing protein 9; WT, wild-type; PAS, periodic acid-schiff; Th1, T helper 1 cell; Th2, T helper 2 cell; MPO, myeloperoxidase; %AREA, the percentage of positive area; MyD88, myeloid differentiation primary response gene 88; PDA, potato dextrose agar; PBS, phosphate-buffered saline; CFUs, colony-forming units; HE, hematoxylin-eosin.

Ethics Statement

Our animal experiments with *Phialophora verrucosa* required an Animal Biosafety Level 2 (ABSL-2) laboratory, as specified by the "Directory of Pathogenic Microorganisms Infectious to Humans" from China's National Health Commission. Since our institution's animal facility lacks ABSL-2 capabilities, all experiments were conducted at Shanghai Yishang Biotechnology Co., Ltd., which provides certified ABSL-2 facilities. The animal study protocol was approved by the Ethics Committee of Shanghai Yishang Biotechnology Co., Ltd. (protocol code IACUC-2023-Mi-204 and date of approval 2023-08-03) in compliance with the Chinese national standard GB/T 35892-2018 "Laboratory animal - Guideline for ethical review of animal welfare".

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Author Contributions

All authors made a significant contribution to the work reported, whether in the conception, review design, execution, acquisition of data, analysis, and interpretation, or in all these areas, took part in drafting, revising, or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

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Disclosure

The authors report no conflicts of interest in this work.

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