

# *Talaromyces marneffi* Can Capture CD86 Proteins of Macrophages in vitro

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**Background:** *Talaromyces marneffi* (*T. marneffi*) is a thermally dimorphic fungus endemic to Southeast Asia that causes human systemic infection. Our earlier immunohistochemical studies revealed that the organisms were markedly labeled with the CD86 antigen in cutaneous lesions brought on by infection. However, the relationship between *T. marneffi* and the CD86 co-stimulatory molecule is still unknown.

**Objective:** To explore the association between CD86 Protein and *Talaromyces marneffi* organisms in vitro and discuss the potential mechanisms.

**Methods:** We created the CD86-EGFP fusion protein in THP-1 macrophages and co-cultured *T. marneffi* conidia with it. We used confocal fluorescence microscopy to view in vitro dynamics. The link between CD86 Protein and *Talaromyces marneffi* organisms in vitro was discovered using immunoelectron microscopy, indirect immunofluorescence test, and immunohistochemistry assay.

**Results:** *T. marneffi* cells received soluble CD86-EGFP from THP-1 macrophages detected by confocal fluorescent microscopy. Both the indirect immunofluorescence assay and the immunohistochemical assay showed that *T. marneffi* conidia were stained by the CD86 marker. Immunoelectron microscopy showed that characteristic colloidal gold particles were observed in *T. marneffi* organisms when co-cultured with THP-1 macrophages.

**Conclusion:** *T. marneffi* organisms have the ability to capture CD86 proteins from macrophages in vitro.

**Keywords:** *Talaromyces marneffi*, macrophage, CD86, confocal microscopy, immunoelectron microscopy, immunohistochemistry

## Introduction

*Talaromyces marneffi* (*T. marneffi*), formerly called *Penicillium marneffi*, is endemic in Southeast Asia and Southern China. It is a thermally dimorphic fungus and an intracellular, opportunistic pathogen that causes disseminated fungal infection and death in people with Human Immunodeficiency Virus/Acquired Immunodeficiency Syndrome (HIV/AIDS) as well as in immunocompromised individuals.<sup>1,2</sup> The incidence of infection has been found to be decreasing with the regular use of antiretroviral therapy and antifungal drugs in patients with HIV/AIDS. However, in secondary immunocompromised individuals or those traveling to these endemic regions, infection has been found to be increasing.<sup>3,4</sup> Upon surviving in macrophages, the conidia can change into yeast and travel to the skin and internal organs. It interacts with macrophages and stimulates host macrophage polarization to enhance expression of M1/M2 type markers.<sup>5</sup> IFN- $\gamma$  is also necessary for the activation of macrophages and the formation of granulomas during infection.<sup>6</sup> In individuals who were HIV-negative, autoantibodies to IFN- $\gamma$  were the primary source of severe infections in those areas.<sup>7</sup> Despite antifungal therapy, the mortality rate of infection is higher than most HIV-associated complications in patients with HIV,<sup>8</sup> which is up to 33.3% in Vietnam and 13.3% in China,<sup>9,10</sup> and is up to 29.4% in non-HIV-infected people.<sup>11</sup>

Uncertainty surrounds the process by which it survives in host cells, and this survival is crucial for preventing its pathogenicity from spreading widely. In a previous study, we used immunohistochemistry to discover that the CD86 marker stained the yeast organisms in cutaneous lesions of patients with infection.<sup>12</sup> Furthermore, our study showed that

CD86 expression of macrophages was significantly decreased when THP-1 cells were co-cultured with conidia for 72 h.<sup>13</sup> The association of CD86 protein with organisms merits investigation into whether it has the ability to bind soluble CD86 or whether the organisms have cross reactivity with CD86 antibody. In this study, we constructed the fusion protein CD86-EGFP in THP-1 macrophages with a lentivirus vector and tried to discover the mechanism of organisms affecting the CD86 protein of macrophages.

## Materials and Methods

### Macrophage Cells and Fungal Strains

The human monocytic cell line, THP-1 cell, used in this study was purchased from the Cell Bank, Chinese Academy of Sciences. THP-1 cells were cultured in Roswell Park Memorial Institute medium (RPMI 1640, Gibco, Thermo Fisher, USA) supplemented with 10% heat-inactivated fetal bovine serum (Gibco, Thermo Fisher, USA) and 1% penicillin-streptomycin solution (Meilunbio, China). THP-1 cells were differentiated into macrophages after induction of 50ng/μL PMA (Meilunbio, China) for 48 hours. Following induction with 100 ng/mL LPS (Meilunbio, China) and 20 ng/mL IFN-γ (Novoprotein, China) for 24 hours, macrophages differentiated into M1-like macrophages. The M1-like macrophages were cultured with Dulbecco's modified eagle medium (DMEM, Gibco, Thermo Fisher, USA), containing 10% FBS and antibiotics (1% penicillin and 1% streptomycin). Peripheral blood mononuclear cells (PBMCs) were isolated from infectious patients and cultured in RPMI 1640.

A strain was isolated from the cutaneous lesion of a patient with talaromycosis admitted as an inpatient to the First Affiliated Hospital of Guangxi Medical University (Nanning, China). It was identified by morphological identification and ITS rDNA sequence analysis. *T. marneffeii* conidia were activated at 27°C on Sabouraud Dextrose Agar for 7–14 days and were grown on Potato dextrose agar for 7 days. The conidia were then harvested and suspended in PBS and counted on the Neubauer improved hemocytometer (Marienfeld, Germany). The conidia were stored at 4°C before use. A *Cryptococcus neoformans* strain was isolated from the cerebrospinal fluid of a patient with cryptococcosis admitted as an inpatient to the First Affiliated Hospital of Guangxi Medical University. It was identified by microscopy and morphological identification of culture on medium.

### Construction of the Fusion Protein CD86-EGFP in THP-1 Cells

The fusion protein CD86-EGFP of THP-1 cells was generated by the lentivirus transfection method. The lentivirus for overexpressing CD86 (LV-CD86-EGFP) and the negative control lentiviruses (LV-NC) were obtained commercially from Genechem Corporation (Shanghai, China). Following the lentivirus construction strategy, human THP-1 cells, which were growing in a logarithmic phase and were cultivated in twelve-well plates, were transfected with LV-CD86-EGFP and LV-NC for 72h respectively. Puromycin (2μg/mL) was added and screened several times. A CD86-EGFP-THP-1 cell line and an NC-THP-1 cell line were constructed. Subsequently, stable CD86-EGFP-THP-1 cell lines were confirmed by quantitative real-time reverse transcription PCR (qRT-PCR) and Western blot.

### Western Blotting Assay

Three groups of THP-1 cells were induced into M1-like macrophages. The cells were collected and lysed by cell lysis buffer including RIPA (Cell Signaling, USA) and 1% protease inhibitor cocktail (Beyotime, China) on ice. The proteins were separated by SDS-PAGE and transferred into a PVDF membrane and sealed with 5% skim milk. Then, the blots were incubated with the primary antibodies, including anti-CD86 (1:1000) and anti-beta Actin (1:10,000) overnight at a 4°C shaker, and the secondary antibody, Goat Anti-Rabbit IgG H+L (HRP) from Engibody (1:10,000) at RT for 1 h. All the above primary antibodies belong to Abcam Corporation from England. The blots were scanned by the Odyssey CLx Infrared Imaging System (LI-COR Biosciences), and the collected pictures were processed by Image Studio Version 5.2 software (LI-COR Biosciences). The experimental groups were performed in triplicates.

## Real-Time Reverse Transcription PCR Analysis

Total cellular RNA of CD86-EGFP-THP-1 cells (OE group), NC-THP-1 cells (NC group), and THP-1 cells (CON group) was extracted by using the RNA prep Pure Cell Kit (TIANGEN, China). Then, RNA was reversely transcribed to cDNA according to the manufacturer's instructions of the Prime Script™ RT Reagent kit (TIANGEN, China). Gene expression was tested with a Q5 Gradient Real-Time PCR Detection System (Bio-Rad, USA) with the SYBR Green System (SYBR qPCR SuperMix Plus, TIANGEN, China). The values were normalized to the GAPDH expression. The experiments were repeated three times.

## Cell Infection and Confocal Microscopy

*T. marneffei* conidia were pre-stained with cell membrane DIL-dye. A model of in vitro infection of CD86-EGFP-THP-1 cells was constructed for our research. M1 polarization of CD86-EGFP-THP-1 cells was induced by treatment with PMA, LPS, and IFN- $\gamma$  for 24 hours. CD86-EGFP-THP-1 cells were infected with conidia at a multiplicity of infection (MOI) of 5 at 37°C for 12 hours, 24 hours, and 48 hours on confocal Petri dishes, respectively. Conidia cultured without THP-1 cells were used as a control group. On the other hand, we performed CD86-EGFP-THP-1 cells co-cultured with heat-killed conidia and *C. neoformans*, respectively. The cell infection was observed under the confocal microscope (Leica TCS SP8, Germany).

## Immunofluorescence Staining

M1 polarization of THP-1 cells (CON group) was infected with conidia at a multiplicity of infection (MOI) of 5 at 37°C for 24 hours. Conidia cultured without THP-1 cells were used as a control group. The cells were fixed with 4% paraformaldehyde for 20 mins at room temperature. The samples were blocked with 5% goat serum for 20 mins at room temperature and incubated overnight at 4°C with mouse anti-B7-2/CD86 (NOVUS, USA) in the refrigerator. Following this, the samples were stained with a second antibody (Coralite488-conjugated Goat Anti-Mouse IgG (H+L), Proteintech, China) for 1h at room temperature. Nuclei were counterstained with 1 $\mu$ g/mL DAPI (Beyotime, China). The staining was observed under the confocal microscope (Leica TCS SP8, Germany).

## Immunoelectron Microscopy

CD86-EGFP-THP-1 cells were infected with conidia at a multiplicity of infection (MOI) of 5 at 37°C for 24 hours. The cells were fixed with a mixture of 4% paraformaldehyde and 0.25% glutaraldehyde, washed with Electron Microscopy buffer, and dehydrated gradually with different acetone concentrations. After dipping in the mixture of acrylic resin and pure acetone and soaking in the acrylic resin mixture for 16 hours, cells were ultra-thin sectioned to a thickness of 50 nm using the ultra-microtome Leica EM UC7. About 1% H<sub>2</sub>O<sub>2</sub> was added for cell permeability, and then the cells were washed with double-steamed water. Cells floated on fetal bovine serum for 30 minutes at room temperature and were washed with PBS containing 1% FBS. The cells were incubated for 24 h at 4°C with mouse monoclonal primary antibody CD86 (B7-2/CD86 antibody, NOVUS, USA) for 60 min, and secondary antibody (goat anti-mouse IgG (H+L) conjugated with 6-nm colloidal gold, Jackson ImmunoResearch, USA) for 30 min. Lastly, the images were observed and collected under a transmission electron microscope (HT7800, Hitachi, Japan). Conidia cultured without THP-1 cells were used as a control group.

## Immunohistochemistry Assay

At a multiplicity of infection (MOI) of 5, conidia were inoculated into M1 polarized CD86-EGFP-THP-1 cells (OE group), THP-1 cells (CON group), and PBMCs for 24 hours each at 37°C. The cells were digested with 0.25% trypsin for 5 minutes at room temperature. Cells were collected by centrifugation and were smeared on glass slides. They were fixed with 4% paraformaldehyde for 20 minutes at room temperature and blocked with goat serum at room temperature for 20 minutes to prevent non-specific conjunction. The cells were incubated with primary antibody (Rabbit Anti-CD86 antibody, Bioss, Beijing, China) overnight at 4°C, biotin goat-anti-rabbit IgG (Bioss, China) for 20 min at room temperature and Horseradish enzyme-labeled streptomycin working solution for 20 min at room temperature. Conidia

cultured without THP-1 cells was used as a control group as well as no primary control was performed (same protocol was followed as above, except the sample was not incubated with CD86 antibody). The experiments were repeated three times.

## Statistical Analysis

Statistical software SPSS 22.0 was used for the analysis. The measurement data was shown as mean  $\pm$  standard deviation ( $X \pm SD$ ). A Student's *t*-test was used for comparing between groups, and a one-way analysis variance (ANOVA) was used for three-group comparisons.

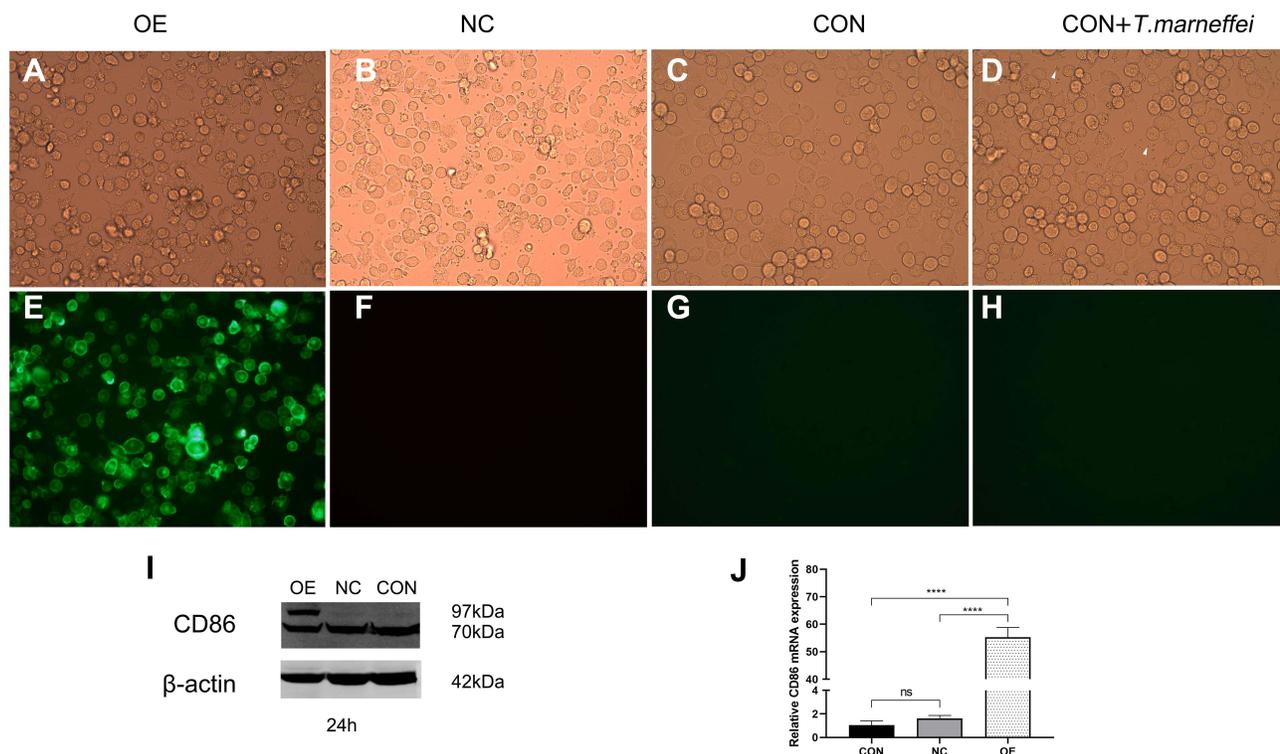
## Ethics Statement

The studies involving human participants were reviewed and approved by the Ethics Committee of the first affiliated hospital of Guangxi Medical University, approval number (KT-004). The patients provided their written informed consent to participate in this study. This study was performed in accordance with the guidelines outlined in the Helsinki Declaration.

## Results

### Lentivirus-Mediated Fusion Protein CD86-EGFP Overexpression in THP-1 Cells

The morphology of CD86-EGFP-THP-1 cells (Figure 1A) had no significant difference in comparison with THP-1 cells of the NC group (Figure 1B) and CON group (Figure 1C). The CD86-EGFP-THP-1 cells appeared to have strong GFP-positive signals after transfection LV-CD86 (Figure 1E), but there was no green fluorescence in the NC group (Figure 1F)

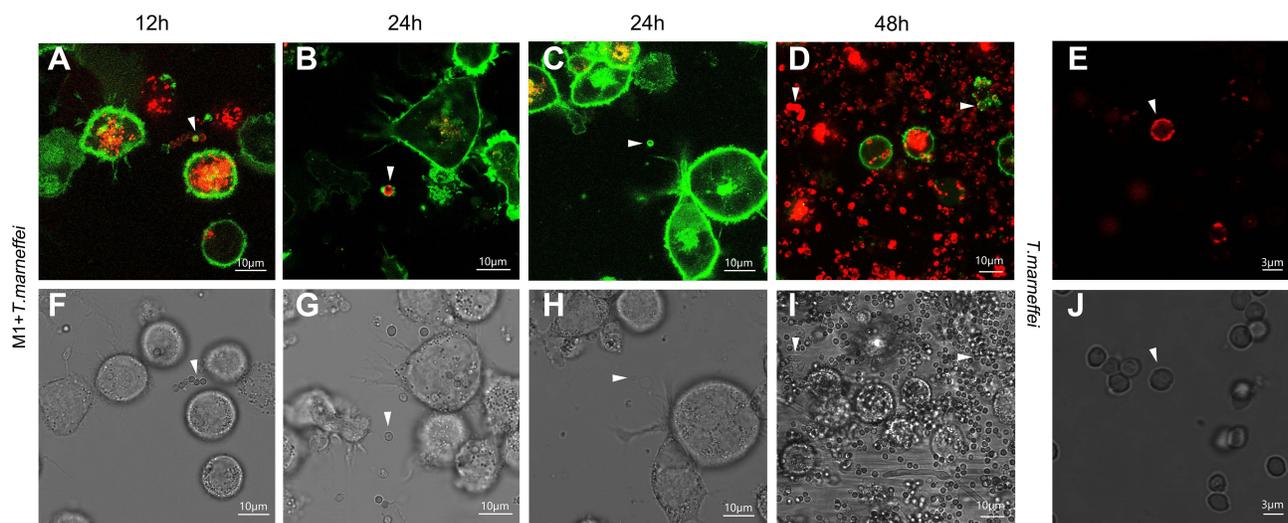


**Figure 1** CD86-EGFP expression in THP-1 cells via lentivirus. (A) CD86-EGFP-THP-1 cells of the OE group, (B) NC-THP-1 cells of the NC group, (C) THP-1 cells of the CON group and (D) co-culture of *T. marneffeii* with macrophages of the CON group (the white arrows showed *T. marneffeii* conidia) were observed under light microscopy. (E) The CD86-EGFP with green fluorescence appeared in the OE group, but no green fluorescence appeared in (F) the NC group, (G) the CON group and (H) the macrophages of the CON group after *T. marneffeii* infection. (I) The specific CD86 protein bands of the NC and CON groups appeared at 70 kDa, and the specific protein bands of the OE group appeared at 70 kDa and 97 kDa. The band of EGFP was approximately 27 kDa, so the band at 97 kDa represented fusion protein CD86-EGFP. (J) The CD86 expression was significantly increased in the OE group in comparison with the NC group and CON group, respectively, by qRT-PCR analysis (\*\*\*\* $P < 0.0001$ , ns  $P > 0.05$ ) (original magnification:  $\times 400$ ).

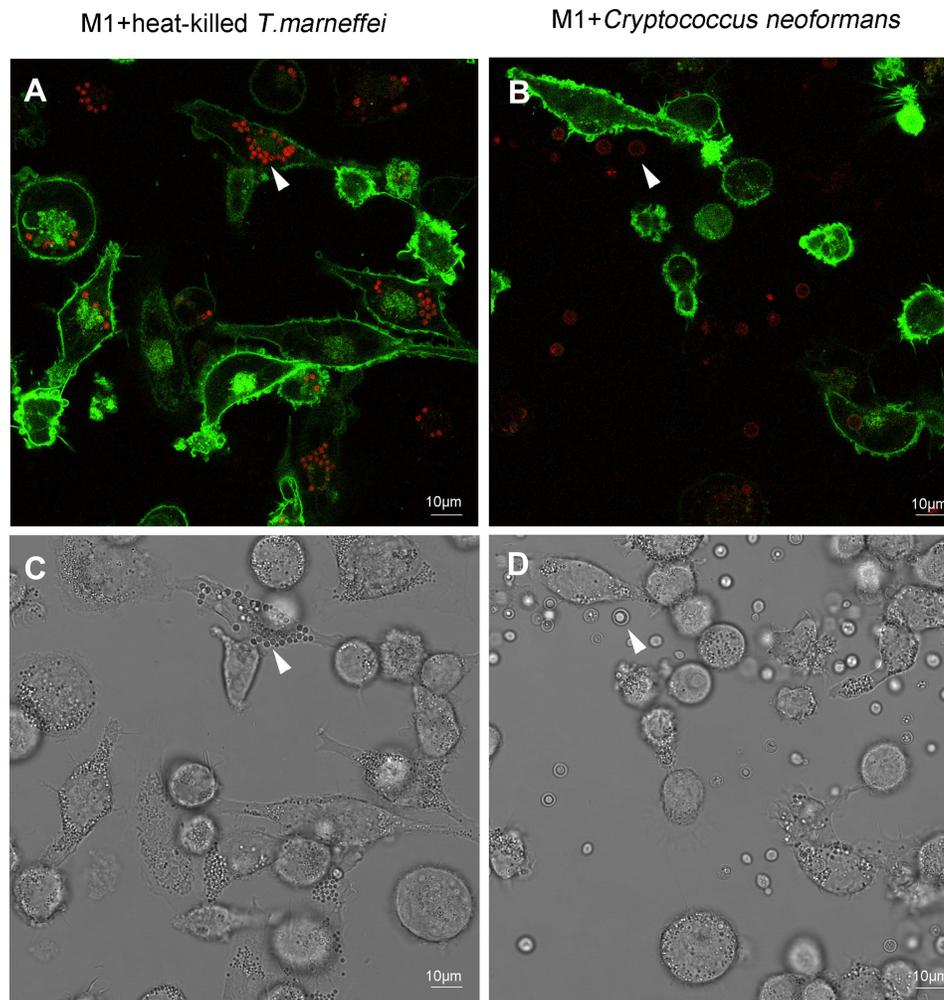
and CON group (Figure 1G). The fusion protein CD86-EGFP band of the OE group was shown by Western blotting (Figure 1I). It demonstrated that the fusion protein CD86-EGFP was expressed in THP-1 cells and that a CD86-EGFP-THP-1 cell line could be successfully created. The qRT-PCR results indicated that there was a significant CD86 overexpression in CD86-EGFP-THP-1 cells in comparison with the cells of the NC group and the CON group. There was no difference in CD86 expression between the NC group and the CON group (Figure 1J). In addition, we have confirmed that THP-1 macrophages of the CON group appeared no fluorescence after TM infection observed by fluorescence microscopy (Figure 1D and H). The experiments were repeated three times.

## *T. marneffei* Conidia Bind the CD86-EGFP Protein Produced by CD86-EGFP-THP-1 Cells

In order to track CD86 protein transfer from macrophages, our approach in this study was similar to that used in the study of trans-endocytosis of CD80 and CD86 between antigen presenting cell and CTLA-4+ cells.<sup>14</sup> We constructed the CD86-EGFP-THP-1 cell line and the cells were co-cultured with conidia. Confocal microscopy imaging of live cells revealed that CD86-EGFP was present on both the cell membrane and the cell nucleus, with the cell membrane being the main site of appearance. Additionally, the presence of numerous green dots all over the CD86-EGFP-THP-1 cells suggested that the cells were secreting CD86 protein. In addition, conidia co-cultured with CD86-EGFP-THP-1 cells by consecutive time-lapse imaging of confocal microscopy showed that CD86-EGFP protein tightly stuck to conidia and they moved together. DiI-dye labeled the conidia, which looked like signet ring cells (Figure 2A), and green dot-shaped particles stuck to the surface of the conidia (Figure 2B). Some of the conidia binding CD86-EGFP appeared as an inclusion body-like unstained structure (Figure 2C) that we observed in a previous study by CD86 immunohistochemistry stain.<sup>12</sup> The conidia were confirmed under bright field microscopy (Figure 2F-H). Those characteristic appearances of conidia binding CD86 were all observed at 12h-coculture (Figure 2A), 24h-coculture (Figure 2B and C), and 48h-coculture (Figure 2D) in the CD86-EGFP-THP-1 group, but they did not appear in the control group (Figure 2E). Furthermore, it was obvious for the proliferation to be discovered, followed by the appearance of the fission yeasts. Meanwhile, there was a decrease in the activity of CD86-EGFP-THP-1 cells, with fluorescence intensity decreasing



**Figure 2** Live-cell imaging of co-culture of *T. marneffei* with CD86-EGFP-THP-1 cells by confocal microscopy. CD86-EGFP appeared with green fluorescence predominantly distributed on the cell membrane of THP-1 cells. Some of the conidia bound CD86-EGFP on the surface of the organisms. (A) *T. marneffei* conidia were labeled with cell membrane DiI-dye, which appeared with red fluorescence at 12 h. (B) At 24 hours, CD86-EGFP appeared as green dot-shaped particles stuck to the surface of the conidia. (C) At 24 hours, the conidia binding CD86-EGFP appeared with a body-like inclusion. (D) The conidia bind CD86-EGFP proteins, as indicated by the arrow in the top right corner. The proliferation of *T. marneffei* to be discovered, followed by the appearance of the fission yeasts (the arrow in the top left corner showed) at 48 h. Meanwhile, a decrease in the activity of macrophages was observed, with fluorescence intensity decreasing significantly. (E) *T. marneffei* conidia were cultured without macrophages. No green fluorescence was observed. (F) The image of A observed under a bright field. (G) The image of B observed under a bright field. (H) The image of C observed under a bright field. (I) The image of D observed under a bright field. (J) An image of E taken in a bright light. The white arrow showed *T. marneffei* conidia as the same as appeared in images of (A–E), respectively (original magnification: (A, C, D, I, F, H) ×630; (B, E, G, J) ×1000).

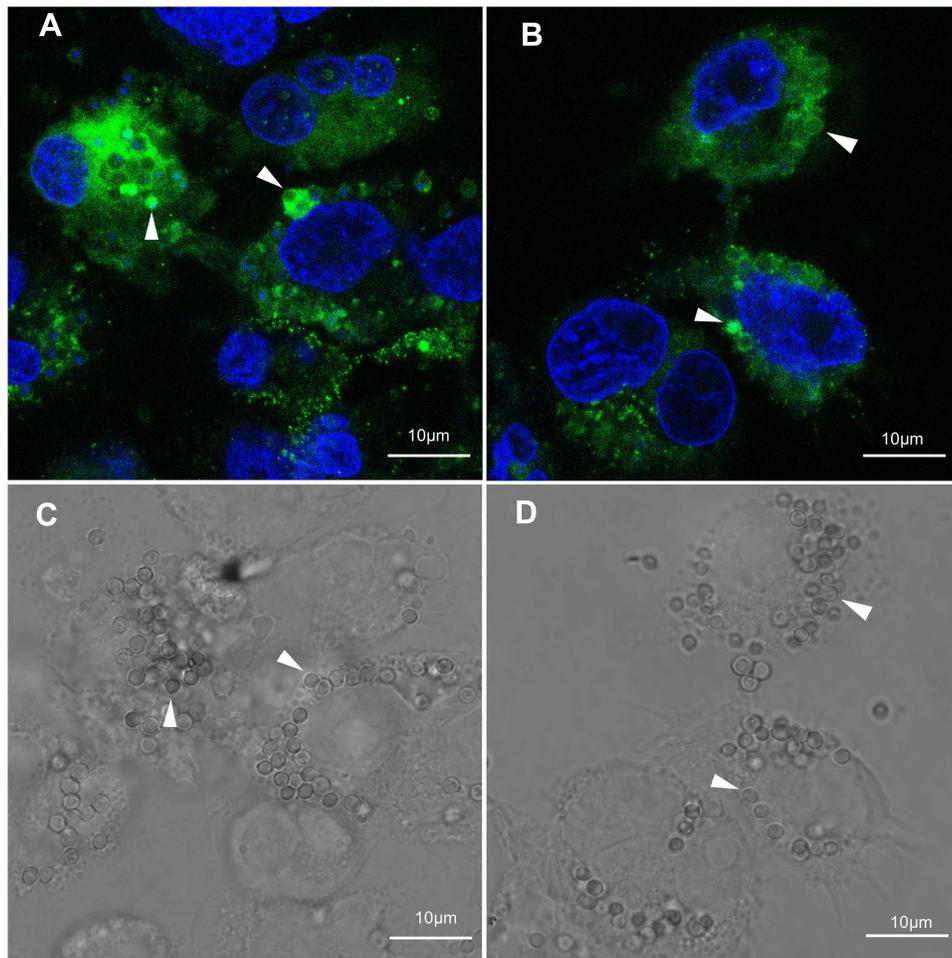


**Figure 3** Live-cell imaging of CD86-EGFP-THP-1 cells infected with heat-killed *T. marneffeii* and *Cryptococcus neoformans* by confocal microscopy. (A) CD86-EGFP-THP-1 cells (green) were incubated for 24 hours with DIL-labeled heat-killed *T. marneffeii* conidia (red). It showed that there was no green fluorescence appearing in the heat-killed *T. marneffeii* conidia. (B) For 24 hours, DIL-labeled *C. neoformans* (red) infected CD86-EGFP-THP-1 cells (green). It showed that *C. neoformans* organisms did not bind the CD86-EGFP protein. (C) The image of A observed under bright field. (D) The image of B observed under bright field (original magnification:  $\times 630$ ).

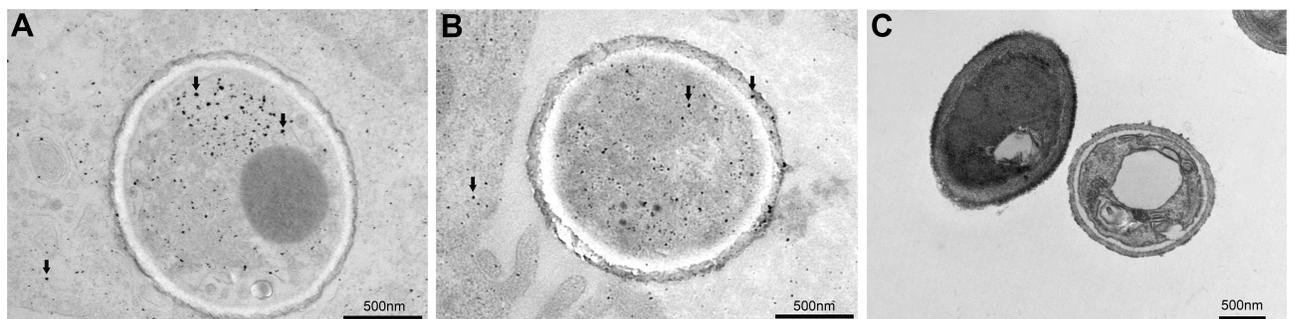
significantly, after 48 hours of incubation (Figure 2D). We did not observe that CD86 proteins bind to heat-killed conidia and *C. neoformans* organisms via confocal microscopy (Figure 3).

### CD86 Immunofluorescence Staining of *T. marneffeii* Conidia Co-Cultured with THP-1 Cells

We also identified conidia co-cultured with THP-1 cells (CON group) by indirect immunofluorescence analysis in order to confirm that conidia had the capacity to bind CD86 protein but not EGFP proteins. It showed that the conidia appeared to have green fluorescence, which indicated that *T. marneffeii* organisms had an ability to bind CD86 protein (Figure 4). Some of the conidia appeared as inclusion body-like unstained structures or looked like signet ring cells by confocal microscopy. We did not observe that conidia appeared with green fluorescence in the control group.



**Figure 4** CD86 indirect immunofluorescence assay of THP-1 (CON group) cells co-cultured with *T. marneffei* conidia by confocal microscopy. **(A and B)** The nucleus of THP-1 cells and *T. marneffei* conidia were counterstained with DAPI (blue). *T. marneffei* conidia were highlighted by green fluorescence, which appeared as signet ring-like cells or inclusion body-like cells. **(C)** The image of A observed under bright field. **(D)** The image of B observed under bright field. The white arrow showed *T. marneffei* conidia as the same as appeared in images of A and B, respectively (original magnification:  $\times 1000$ ).



**Figure 5** TCD86 expression was detected on co-culture of *T. marneffei* conidia with CD86-EGFP-THP-1 cells by immunoelectron microscopy. **(A)** Colloidal gold particles about 6 nm appeared on intracellular *T. marneffei* conidia of macrophage cells. **(B)** Colloidal gold particles of about 6 nm appeared on extracellular *T. marneffei* conidia of macrophage cells. **(C)** There were no colloidal gold particles on *T. marneffei* conidia cultured in medium lacking THP-1 cells (Black arrows show characteristic colloidal gold particles).

## Presentation of Colloidal Gold Particles on the CD86-EGFP-THP-1 Cells and the Conidia

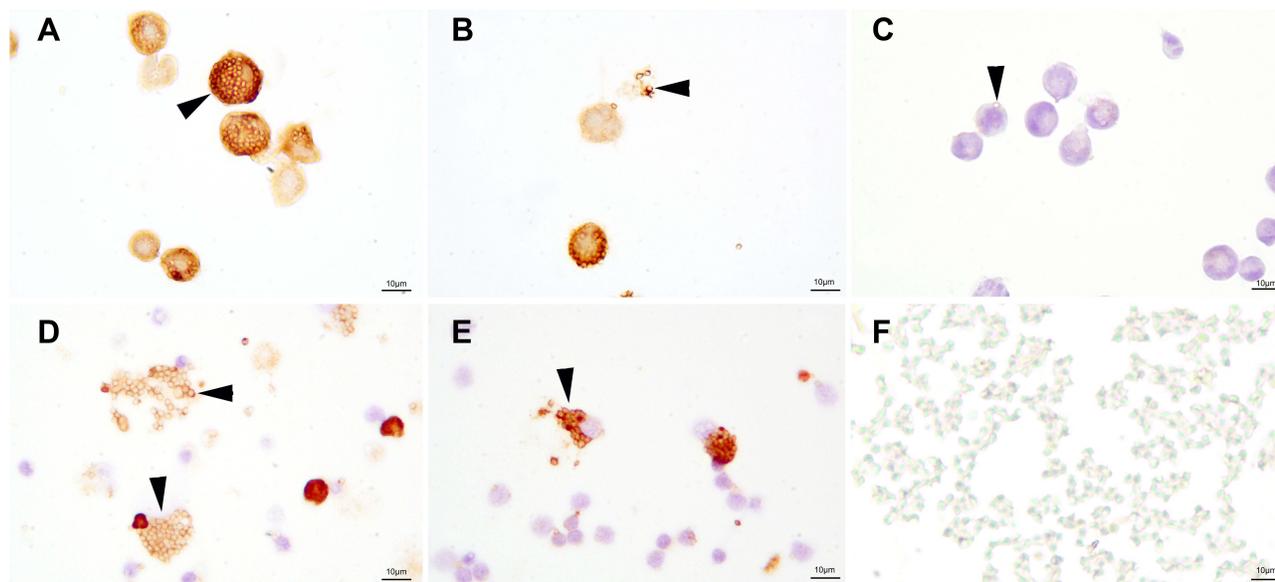
We labeled monoclonal anti-CD86 antibodies using colloidal gold particles that were about 6 nm in diameter. After co-culture of CD86-EGFP-THP-1 cells and conidia, we observed dense electric particles of about 6 nm presented on both the CD86-EGFP-THP-1 cells and the conidia. The dense electric particles appeared on the cell wall and cytoplasm of the conidia regardless of whether the conidia were intracellular or extracellular to macrophage cells (Figure 5A and B). When conidia were cultivated alone without co-culture with THP-1 cells, there were no dense electric particles visible on the conidia (Figure 5C).

## Characteristic CD86 Immunohistochemistry Stain of *T. marneffei* Conidia Co-Cultured with THP-1 Cells and PBMCs

The immunohistochemistry showed that CD86 stained THP-1 cells of the OE group and CON group, as well as macrophage cells in PBMCs (Figure 6A, B, D and E). Numerous tiny ovoid *T. marneffei* conidia were detected inside or outside of macrophages and showed positive CD86 expression. The conidia appeared as a characteristic stain, which looked like signet ring cells or an inclusion body-like unstained structure, resembling the Dutcher body in the plasma cell (Figure 6A, B, D, E). No primary controls showed negative staining of macrophages and *T. marneffei* conidia in the OE group (Figure 6C). When conidia were cultured in medium without macrophage cells, the CD86 stain was negative (Figure 6F).

## Discussion

The immune system's phagocytes provide the first cellular line of defense against pathogens after inhalation of aerosolized conidia. Among them, the activation status of macrophages is critical for their antifungal function. Macrophage polarization status has been activated into two functional phenotypes, including classically activated macrophages (M1) and alternatively activated macrophages (M2). M1-like polarization plays a vital role in the immune response to bacteria and intracellular pathogens and participates in pro-inflammatory responses governed by toll-like receptors (TLRs). M2-like polarization is essential in the immune response to allergy, asthma, and helminth infection and



**Figure 6** CD86 stain of *T. marneffei* conidia co-culture with THP-1 cells and PBMCs by immunohistochemistry. Mounds of *T. marneffei* conidia and macrophage cells had positive expression of CD86. The *T. marneffei* conidia with CD86 stain looked like signet rings. In (A), CD86 stained both *T. marneffei* conidia and THP-1 cells in the OE group. (B) In the CON group, CD86 stained both *T. marneffei* conidia and THP-1 cells. (C) Negative CD86 staining of *T. marneffei* conidia and THP-1 cells in the absence of a primary control. (D and E) *T. marneffei* conidia and scattered cells of PBMCs had positive expression of CD86 when the conidia were co-cultured with PBMCs. (F) Negative CD86 stain of *T. marneffei* conidia cultured without macrophages (original magnification:  $\times 1000$ ).

participates in anti-inflammatory responses dominated by the TH2 response.<sup>15</sup> In the in vivo microenvironment, they coexist and undergo mutual interconversion. Recent studies found that the fungus can adapt to the intracellular macrophage environment, impacting its polarization in vitro.<sup>16</sup> Fungal dimorphic switching has recently been identified as a critical mechanism that allows to avoid host immune responses and reproduce in macrophage niches.<sup>17</sup> Furthermore, according to Wei et al, M2-like macrophages were better suited to the survival of fungus and it could change M1-like macrophages into M2-like ones by downgrading SOCS3 expression and activating the TLR9 pathway.<sup>18</sup> Based on our prior studies, we considered that fungus might bind to the CD86 protein of macrophages and have an effect on M1-like and M2-like transitions by an unknown mechanism.

Several studies discovered that murine macrophage was polarized to M1 at the early stage of infection with *T. marneffeii* in vitro. However, the levels of M1 markers (TNF- $\alpha$ , CD86) decreased while M2 markers (Arg-1, CD206) significantly increased as infection progressed.<sup>19–22</sup> Furthermore, *T. marneffeii* pathogen had a capability of inducing THP-1 macrophages to M2-like polarization.<sup>18</sup> Intriguingly, we discovered using confocal microscopy those CD86-EGFP proteins transferred from THP-1 cells to bind conidia, suggesting that they possess the capacity to capture CD86 proteins. We carried out more assays in order to confirm this new finding. Firstly, colloidal gold particles were used to label monoclonal anti-CD86 antibodies. We discovered using immunoelectron microscopy that conidia co-cultured with CD86-EGFP-THP-1 cells had the characteristic colloidal gold particles on the cell wall and cytoplasm. However, there were no characteristic colloidal gold particles in the control group. These findings indicate that conidia bind to and capture CD86 proteins. Secondly, indirect immunofluorescence showed that conidia were stained by CD86 antibody via secondary fluorescence antibody in the co-culture group but negative in the control group. Thirdly, we found that the characteristic CD86 stain appeared on conidia both in co-culture with several THP-1 cell lines and PBMCs as described in our previous study.<sup>12</sup> In conclusion, these results demonstrated that conidia had an ability to capture CD86 protein from macrophages in vitro. A study demonstrated that the M2 protein encoded by the vaccinia virus binds CD86 and CD80, which blocks their interaction with CD28/CTLA4.<sup>23</sup> We are aware of no other reports that suggest a fungal organism can seize CD86 protein prior to this one. Uncertainty surrounds the mechanism by which CD86 is captured. We hypothesized that some proteins might be able to bind CD86 protein.

CD86, along with CD80, is a co-stimulatory signal commonly expressed on APCs as a ligand for CD28/CTLA-4. Therefore, the expression of CD86/CD80 would impact the activation of the CD4+ T lymphocytes. Activation of CD4+ T lymphocytes plays a prominent role in the elimination of intracellular pathogens. There was evidence that CD4+ T cell activation was impaired in HIV-negative patients with infection.<sup>24</sup> Immunofluorescence and Western blot analysis revealed in our earlier study that THP-1 cells co-cultured with conidia for 72 hours significantly reduced CD86 expression.<sup>13</sup> Therefore, we made the assumption that organisms captured CD86 protein and contributed to CD86 protein exhaustion, which led to a lowering of CD4+ T lymphocyte cell activation. Overall, our research provided a strong foundation to investigate the relationship between the CD86 protein and organisms. Future research is needed to determine how CD86 protein is captured and how this affects T lymphocyte differentiation.

## Conclusion

*T. marneffeii* organisms have the ability to capture CD86 proteins from macrophages in vitro.

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## Disclosure

The study's authors declare that there were no financial or commercial relationships that might be viewed as having a potential conflict of interest.

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