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Active phagocytosis of *Mycobacterium tuberculosis* (H37Ra) by T lymphocytes (Jurkat cells)

Min Zhang[a,b,1], Qi Zhu[a,1], Ming Shi[a], Yang Liu[a], Lei Ma[a], Yining Yang[a], Dongyun Feng[a], Wen Dai[a], Lin Zhang[a], Tao Kang[a], Ping Chen[a], Ying He[a], Tingting Liu[a], Qing Zhao[a], Wenjing Wang[a], Jin Zhi[a], Guodong Feng[a,**], Gang Zhao[a,*]

[a] *Department of Neurology, Xijing Hospital, the Fourth Military Medical University, No. 15 Changle-Xi Road, Xi'an, Shaanxi Province 710032, China*
[b] *Department of Neurology, Fuzhou Dongfang Hospital, No.156 West Second Ring Road, Fuzhou, Fujian Province 350025, China*

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ABSTRACT

This study aimed to co-culture Jurkat T lymphocytes with inactivated *Mycobacterium tuberculosis* (Mtb H37Ra), explore whether T lymphocytes could phagocytose H37Ra cells, and determine the underlying mechanism. Jurkat T lymphocytes were co-cultured with H37Ra cells, and confocal laser scanning microscopy, electron microscopy, and flow cytometry techniques were used to identify phagocytosis and elucidate its mechanism. After Jurkat T lymphocytes phagocytosed H37Ra cells, the cell body became larger, with abundant cytoplasm, the portion of the nucleus closest to the bacterium deformed, long and short pseudopodia were extended, and the folds of the cell membrane formed depressions that created phagocytic vesicles surrounding the bacterium. The macropinocytosis inhibitor amiloride and the cytoskeletal inhibitor cytochalasin D were found to inhibit phagocytic efficacy; serum complements might enhance phagocytosis through opsonization. Jurkat T lymphocytes could actively phagocytose inactivated Mtb via the macropinocytotic mechanism. Actin remodeling played an important role in the macropinocytotic process. Serum complements may regulate phagocytosis.

1. Introduction

Innate immune cells are well known to form the first line of defense in humans; they are known to play a specialized antigen-presenting role and are specialized for phagocytosis. This specialized role in phagocytosis includes endocytosis of larger particles (diameter >0.5 μm), actin polymerization and remodeling, and formation of phagocytosis-phagolysosomes (Krause, 2000; Rabinovitch, 1995). When cells with specialized phagocytic function ingest *Mycobacterium tuberculosis* (Mtb), they can kill Mtb directly via autophagy or the phagolysosome or apoptotic pathways, and can also activate cellular immunity via their antigen-presenting function and secretion of cytokines (Huynh et al., 2011). Because Mtb is an intracellular parasite, the bactericidal effect of tuberculosis antibodies is inhibited, and innate immune system-mediated phagocytosis and acquired immune system-mediated cellular immunity are the main pathways for elimination of Mtb (Lawn and Zumla, 2011; Welin and Lerm, 2012). Coordination between innate and acquired immunity plays an important role in the human immune response.

Traditionally, bone marrow-origin cells, such as monocytes, phagocytes, neutrophils, and dendritic cells, exhibit characteristics of innate immunity (Rabinovitch, 1995). Whereas B lymphocytes are involved in classic acquired immune reactions, T lymphocytes inhibit the proliferation of intracellular Mtb or kill phagocytic cells that are infected by Mtb, mainly through secretion of cytokines and cytotoxic effects, although they have no ability to phagocytose Mtb (Boom et al., 2003; Worku and Hoft, 2003). Thus, little research has been conducted on the phagocytic role of T lymphocytes toward bacteria. However, in-depth studies have recently been conducted on the phagocytic role of lymphocytes, and many laboratories have reported that lymphocytes perform phagocytosis. For example, Wu et al. (2009) reported that T lymphocytes (such as γδT) could simultaneously exhibit their specialized phagocytic functions and

* Corresponding author. Tel.:+86 1083198890/29 84771056.
** Corresponding author at: University, No. 15 Changle-Xi Road, Xi'an, Shaanxi Province 710032, China. Tel.: +86 29 84775361.
E-mail addresses: fgd2000@163.com (G. Feng), zhaogang@fmmu.edu.cn (G. Zhao).
[1] Joint first authors.

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present antigens to αβT CD4+ cells through MHCII. Soon after, García-Pérez et al. (2012) used human Raji B cells to show that B lymphoma cells can actively phagocytose *Salmonella typhi* and Mtb via macropinocytosis. Using a modified acid-fast staining technique, our laboratory also confirmed the presence of Mtb inside CD3+-positive T lymphocytes in the cerebrospinal fluid of tuberculous meningitis patients (Chen et al., 2012). Based on these results, we inferred that T lymphocytes, which have traditionally been considered to be non-phagocytic, have the ability to phagocytose Mtb. We co-incubated human acute T lymphoblastic leukemia (Jurkat) T lymphocytes and inactivated Mtb H37Ra, then used a variety of experimental methods, such as light microscopy, electron microscopy, confocal laser microscopy, flow cytometry, and endocytosis inhibitors to monitor phagocytosis, and confirmed that Jurkat T lymphocytes might phagocytose Mtb via macropinocytosis.

2. Materials and methods

2.1. Jurkat cell line

The human acute T lymphoblastic leukemia Jurkat T lymphocyte line was gifted by the Department of Genetics, the Fourth Military Medical University (American Type Culture Collection, Rockefeller, MD, USA). It was cultured in 10% fetal bovine serum (HyClone, Logan, UT, USA), 90% RPMI-1640 medium (HyClone), and 1% penicillin–streptomycin (10,000 units/ml penicillin and 10,000 μg/ml streptomycin; Sigma, Shanghai, China) at 37 °C, 5% CO_2, and 1 atm atmospheric pressure.

2.2. Fluorescent beads, H37Ra, and bacterial fluorescence labeling

FITC-labeled poly-propylene-ethylene fluorescent beads (Sigma), with a diameter of approximately 1 μm, were used in this study. Heat-inactivated Mtb H37Ra (Becton, Dickinson and Company, Franklin Lakes, NJ, USA) was ground in a *Mycobacterium* bacteria-grinding bottle (Zhuhai Baso Diagnostics Inc., Zhuhai, China) at room temperature under sterile conditions for 1 min. RPMI-1640 was then added in 10% FBS medium for resuspension and allowed to stand for 1 h. Then, the supernatant was collected and the precipitate was discarded, and the bacteria were aliquoted at a concentration of 5×10^7/ml and stored at −20 °C for future use.

The counted and subpackaged bacteria were centrifuged at 8000 × *g*/min for 10 min, and the supernatant was discarded. Sterile carbonate buffer (0.1%, 100 μl, pH 9.6) was added for resuspension, following which 10 μl of 10 g/ml FITC was added for overnight incubation at 4 °C. Cells were washed with sterile PBS 4 times, resuspended in 1 ml of RPMI1640, and stored at −20 °C for future use.

2.3. In vitro co-incubation

Unless specifically stated, Jurkat cells were seeded in 24-well plates at 5×10^5 cells/well, with bacteria or fluorescent beads at 5×10^6/well, and incubated in 1 ml of RPMI1640 culture medium, which contained 10% FBS and 1% penicillin–streptomycin, at 37 °C, 5% CO_2, and 1 atm atmospheric pressure for 4 h. Flow cytometry was performed to detect cell functions, and the incubation time was set according to the relevant experimental design.

2.4. Light microscopy detection of phagocytosis

The cultured cell suspension was centrifuged at 300 × *g*/min for 10 min, washed twice, and resuspended in the above medium. Droplets of the cell suspension were then placed onto poly-lysine coated slides, spread evenly, and fixed in 4% formaldehyde (pH 7.4) at room temperature for 15 min. Then modified acid-fast staining was performed, after washing with distilled water 10. In brief, slides were soaked in 0.3% Triton X-100 for 30 min, and rinsed 3 times in 0.1 M PBS, for 5 min/time. Slides were re-stained with carbol fuchsin for 15 min, decolorized twice in acidic alcohol, for 2 min/time. Nuclei were liner-stained with hematoxylin for 1 min, then photographed under the oil-immersion lens of a microscope at a magnification of 1000× (Leica DM2500, Dresden, Germany) (Fig. 1).

2.5. Actin staining

The cultured cells suspension were centrifuged and slides were prepared using the methods described above. Cells were fixed in 4% paraformaldehyde for 15 min, soaked in 0.1% Triton X-100 at room temperature for 5 min, and washed twice with sterile PBS. Cells were incubated in 1% BSA at 37 °C for 20 min, followed by incubation with 0.4 unit/slice of rhodamine-phalloidin (Invitrogen, Carlsbad, CA, USA) at room temperature for 20 min, and washed with 0.1 M PBS twice. Cells were stained with 0.01% auramine "O" solution (Sigma; 0.01 g auramine "O" was dissolved in 10 ml of 95% ethanol, and 4.5 g of carbolic acid was dissolved in 90 ml of distilled water; these two solutions were then thoroughly mixed and filtrated) at 50 μl/slice at room temperature for 15 min, and decolorized in 3% acidic alcohol for 3 min. The nuclei were liner-stained with Hoechst dye for 15 min, and the phagocytosis of Jurkat T lymphocytes was observed under 60× magnification by using a confocal laser oil microscope (Olympus FluoView™ FV1000, high thousand spike, Japan).

2.6. Transmission electron microscopy

Jurkat T lymphocytes were incubated with H37Ra or fluorescent beads for 4 h. Non-infected Jurkat cells were incubated for the same period and under the same conditions as a negative control. After incubation, the cell suspension was centrifuged at 300 × *g*/min for 10 min and fixed overnight in 3% glutaraldehyde at 4 °C. Then, the cell suspension was washed with 0.1 M PBS three times. After being fixed in 1% osmium tetroxide at 4 °C for 1 h, the cells were dehydrated using an ethanol gradient, embedded in epoxy resin, and allowed to stand at 60 °C for 3 days. Then, the ultrastructural changes in post-phagocytosis Jurkat T lymphocytes were observed under a transmission electron microscope (JEOL JEM-1230 Aichiken, Sutamina, Japan).

2.7. Video microscopy of living cells

Jurkat T lymphocytes (5×10^5 cells/ml) were added to the above culture medium and seeded into confocal culture dishes. A total of 5×10^6 cells/ml of FITC-labeled H37Ra were then added, and phagocytosis of Mtb by lymphocytes was dynamically observed using the living cell workstation (Olympus FluoView™ FV1000, high thousand spike, Japan) (37 °C).

2.8. Staining of lysosomal acid phosphatase

Slices prepared using the above-described methods were fixed in 4% paraformaldehyde at room temperature and dried for 1 h, and then stained according to the instructions of the acid phosphatase staining kit (Sigma Acid Phosphatase, Shanghai, China). Intracellular lysosomal changes following phagocytosis were then observed under the bright field channel of a 100× oil microscope. A fluorescence microscope was used to synchronously capture images of the