Journal of Public Health & Environment (2024)

Journal of Public Health & Environment

journal homepage: www.journal-phe.online

Paper Type: Original Article

The impact of MRSA on macrophage infiltration and polarization in mouse lung tissues

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Abstract

Objective: To establish a model of methicillin-resistant Staphylococcus aureus (MRSA) pneumonia and observe the effect of MRSA on macrophage polarization. Methods: The MRSA pneumonia model is established by adding 1×108CFU/mL MRSA bacterial solution to BALB/c mice through nasal drip. To make pathological specimens of lung tissue and observe the inflammation of the lungs;to detect the expression of F4/80, CD11B, CD206, and CD86 in mouse lung tissue through flow cytometry to reflect the polarization of macrophages and their M1/M2.

Result: Compared with the uninfected group, macrophages have increased significantly in infected group; The expression of CD206 in macrophages has significantly enhanced after infection, indicating the macrophage polarization towards M2;

Conclusion: MRSA pulmonary infection leads to a significant infiltration of macrophages in lung tissue and their polarization towards the M2 phenotype.

Keywords: Methicillin resistant staphylococcus aureus; Pneumonia; Macrophage polarization; M2 macrophage

1 | Introduction

Staphylococcus aureus(expressed as S. aureus following) is a notorious pathogen capable of causing severe infections in both humans and animals. Owing to its potent pathogenicity and

virulence, it can induce a range of infections, affecting areas such as the skin, soft tissue, cardiovascular system, bones, and internal organs[1]. Before the discovery of penicillin, the mortality rate associated with S. aureus was extremely high. Since the 1940s, the advent of penicillin and its subsequent clinical application have effectively mitigated the harm caused by Staphylococcus aureus infections. However, with the prolonged use of penicillin, strains of S. aureus that are resistant to penicillin have increasingly emerged in clinical settings. The penicillin enzyme it produces can break down the β-lactam ring of penicillin[2]. Consequently, scientists have developed a semi-synthetic penicillin, methicillin, to counteract penicillinase. The introduction of methicillin effectively managed penicillin-resistant S. aureus; Unfortunately, methicillin-resistant S. aureus (MRSA) rapidly emerged clinically. The resistance of MRSA to methicillin and β-lactam antibiotics is confirmed to be associated with the mecA gene[3]. Moreover, prolonged antibiotic therapy in clinical settings has escalated the threat of drug-resistant S. aureus, making its diagnosis and treatment a significant challenge. The gold standard for diagnosing bacterial infections in clinical practice is the positive blood culture for pathogens, yet the blood culture process is lengthy and the early detection rate is low. For patients with respiratory infections, early diagnosis and treatment are crucial. In clinical practice, vancomycin is the drug of choice for MRSA treatment[4]. However, reports of vancomycin-resistant S. aureus are frequent at present[5]. To prevent the emergence of drug-resistant strains, innovative treatment strategies such as combination therapy, photochemical therapy, local ozone therapy, and bacteriophage therapy are continuously being developed. While there are still very few widely and effectively applied treatment methods for respiratory infections in clinical practice. Therefore, developing a stable animal model of MRSA pneumonia is crucial for observing the progression and changes of MRSA pneumonia, studying its pathophysiology, and its treatment and prevention.

Macrophages, as a crucial component of the immune response, are primarily differentiated from mononuclear cells that are originated from the bone marrow[6]. They are widely distributed throughout the body and involved in a wide range of physiological and pathological processes, notably in inflammation, tumor, and tissue repair. In the innate immune response, macrophages eliminate invading pathogens through phagocytosis and also present pathogen antigens to activate adaptive immunity by transmitting immune signals. They can also be activated into M1/M2 macrophages to participate in the regulation of adaptive immunity.

M1 and M2 macrophages represent two extremes. M1 macrophages displaying pro-inflammatory features in the immune response, including the release of inflammatory factors and inducing the accumulation of other immune cells at the inflammatory site to combat pathogens; while M2 macrophages exhibit anti-inflammatory features, including phagocytosis of apoptotic cells and the release of anti-inflammatory factors to promote tissue recovery. Polarization imbalances between M1 and M2 macrophages are often linked to various diseases or inflammations. Understanding macrophage polarization and changes in associated cytokines in diseases involving

macrophages is critical for defining disease progression and informing therapeutic interventions. Macrophage polarization is reflected in alterations of cellular molecular markers and expression. For instance, M1 macrophages overexpress markers such as CD80, CD86, and CD38 on their membrane surfaces[7]. In M2 macrophages, there is heightened expression of Arg-1, CD206, and CD163[8]. Consequently, various macrophage types can be distinguished by detecting specific biomarkers on them. Researches of macrophage polarization in MRSA-induced pneumonia remains scarce. This study establishes a simple but reliable mouse model for MRSA pneumonia and employs flow cytometry to delve deeper into macrophage polarization.

2 | Materials and Methods

2.1 | Construction of MRSA pneumonia mouse model

The experimental animals were SPF BALB/c mice (aged 6-8 weeks). The mice were reared in the animal room of the School of Pharmacy, Anhui Medical University. The feeding process was conducted strictly in accordance with the operating standards for experimental animals of Anhui Medical University. The experiments were performed in the animal laboratory of the School of Pharmacy, Anhui Medical University. After 3 days of adaptive feeding, mice were administered with 50μL of 1×108CFU/mL MRSA bacterial solution via nasal drip for the aim of establishing the MRSA pneumonia model. Bacterial solution preparation: The strains were clinically isolated by the clinical laboratory of Anhui Provincial Children's Hospital, and its type was identified as MRSA ST239. The strains were detached from the culture dish and placed into 20ml TBS liquid medium in a clean environment. After constant-temperature oscillation culture at 37℃ for 24 hours, 1ml of the bacterial solution was taken into fresh TBS liquid medium for constant-temperature oscillation culture at 37℃ for 24 hours. The concentration of the bacterial solution was measured by a cell counter and diluted to 1×108CFU/mL. Animal model construction: The infected group was given 50μL of MRSA (1×108CFU/ml) bacterial solution, and the uninfected group was given 50μL of sterile normal saline solution. The bacterial solution or normal saline was dripped into one side of the mouse's nasal cavity, and after observing the mouse's nasal scratching, the mouse was kept upright and the head was raised for 1 minute to ensure that the bacterial solution could enter the mouse's lungs as much as possible. Continue feeding after completing the nasal drip.

2.2 | MRSA detection of lung tissue

Took mice infected with nasal drops, euthanized them, took fresh lung tissue, ground it into lung tissue homogenate, and placed the lung tissue homogenate on a Columbia blood plate containing penicillin at 37 ℃ for 24 hours to observe the formation of colonies or hemolytic rings.

2.3 | Preparation of lung cell suspension

On the third, fifth, and seventh days after sacrificing the infected mice, lung tissue was collected and stored in PBS solution at low temperature, and ground in a 70-mesh cell filter. The filtered abrasive liquid was transferred to a centrifuge tube, filled to a volume of 20 ml, and supplemented with collagenase I for digestion and decomposition at 37℃ for 1 hour. The digestion and decomposition process was halted in an ice bath for 5 minutes, and the filtered suspension was re-filtered using a 70-mesh filter. The re-filtered suspension was centrifuged at high speed (2200 r, 5 minutes at 4℃) with the supernatant discarded; and then the centrifuged precipitate was added with 1 ml of red blood cell lysate, followed by destruction of red blood cells in the ice bath for 5 minutes. High-speed centrifugation (2200 r, 5 minutes at 4℃) was conducted again to remove the red blood cells with the supernatant discarded. After the centrifuge precipitate was suspended with 1 ml of PBS solution with the supernatant discarded This procedure was repeated twice. Once re-suspended with 1 ml of PBS solution, the cell concentration was counted by a cell counter and adjusted to 1×106/100 CFU for measurement.

2.4 | Flow cytometry

Cell surface marker labeling: Took prepared cell suspension, added Zombie NIR antibody and incubated at 4 ℃ for 30 minutes to distinguish between living and dead cells. Then added CD16/32 and incubated at 4 ℃ for 30 minutes to block non-specific staining of Fc with CD16/32 antibody. Added an appropriate amount of F4/80-Percy/Cy5.5, CD11B-FITC, and CD86-PE and incubated at 4 ℃ for 30 minutes in dark place. Intracellular marker labeling: Took the cell suspension that has completed cell surface marker labeling, added an appropriate amount of PBS for centrifugation followed by fixing and membrane break. After breaking the membrane, added CD206-APC and incubated at 4 ℃ for 30 minutes in dark place to complete intracellular antibody labeling. After completing the marker labeling, the number and differentiation level of macrophages were detected by a flow cytometer CytoFLEX.

2.5 | Data processing

Flow cytometry data were processed by FlowJo, and statistical data were plotted by GraphPad Prism analysis via t-tests. $P<0.05$ indicates that the difference is statistically significant.

3 | Results

3.1 | After infection, the inflammation in the lungs of mice gradually worsened

Pathological section observation: Mice were euthanized on the third, fifth, and seventh day of infection, and lung tissue was taken, embedded in paraffin, and stained with HE to make pathological sections. Observed the structural changes of lung tissue in mice after infection under an optical microscope. Compared with the uninfected group (Figure 1a), on the third day

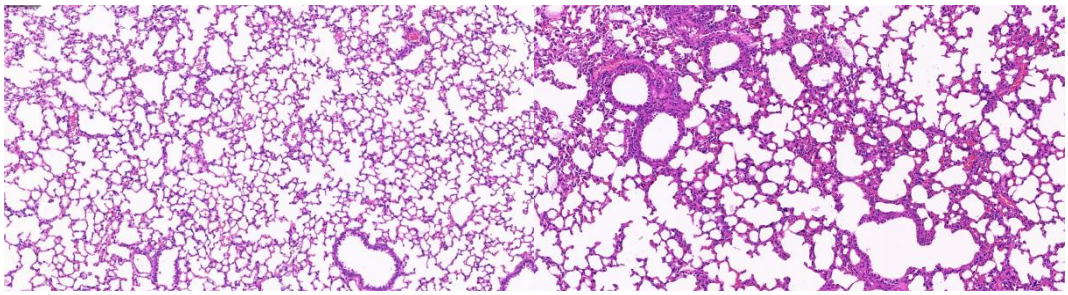
after infection, inflammation infiltration and exudation began to appear in the lungs of mice, and the alveolar wall thickened (Figure 1b). On the fifth day, the infiltration and exudation of lung inflammation worsened, and the alveoli were extensively damaged (Figure 1c). On the seventh day, the lungs were filled with inflammation and consolidation appeared (Figure 1d). The pathological section results indicate that the inflammation of the lungs in mice gradually worsens after infection. Observation of experimental animals showed that compared with the uninfected group, the infected group mice showed a significant decrease in vitality during the subsequent experimental feeding process, and their intake of feed and water decreased compared to the blank control group, which is consistent with the pathological results.

3.2 | After infection, a large amount of macrophages infiltrated the lungs of mice

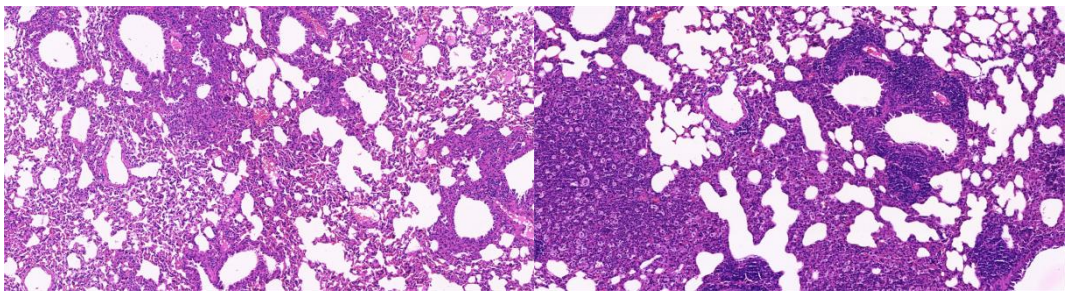
Mice infected with MRSA strains were euthanized at different time points, and lung tissue suspensions were prepared from their lungs. The expression of macrophages (F4/80, CD11b) in the suspensions was detected by flow cytometry. The results showed that compared with the uninfected group, the infected group of mice showed a significant increase in macrophages, which continued to increase over infection time. After MRSA infection, the infiltration of macrophages in lung tissue increased (Figure 2) ($P<0.05$).

3.3 | After infection, macrophages in the lungs of mice polarize significantly towards M2

Detected lung tissue suspensions at different time intervals after infection by flow cytometry. When M1 macrophages highly expressed CD86, M2 macrophages highly expressed CD206 Compared with the uninfected group, the expression of CD206 in macrophages of the infected group significantly enhanced, while the expression of CD86 didn't show a significant upward trend. After MRSA infection, the expression of M2 macrophages in the polarization of pulmonary macrophages enhanced.



Uninfected group（a） 3d（b）



5d（c） 7d（d）

100μm；

Fig1 HE staining sections of mouse lung tissue

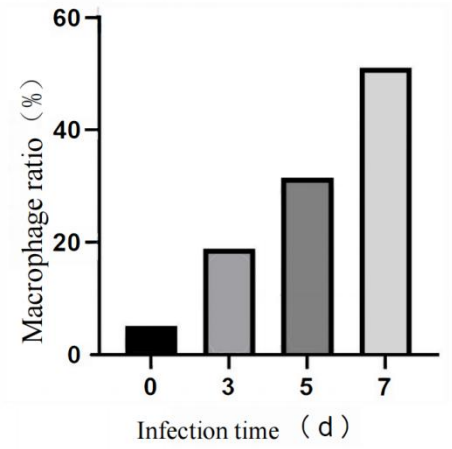
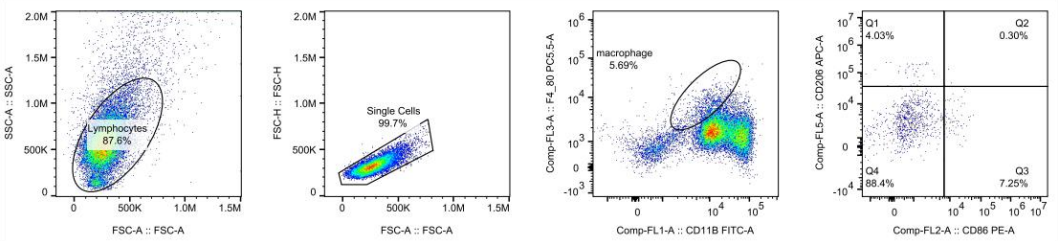
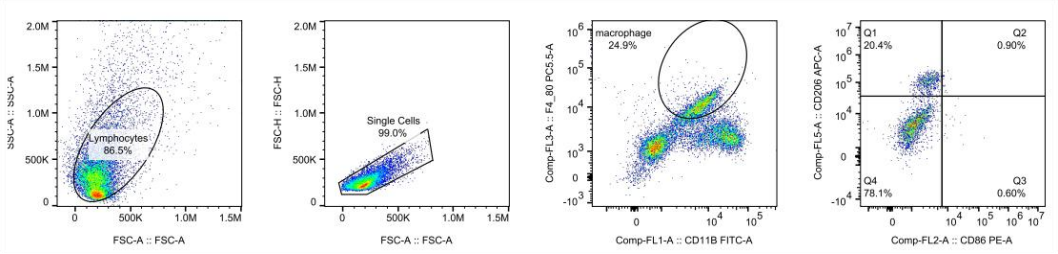


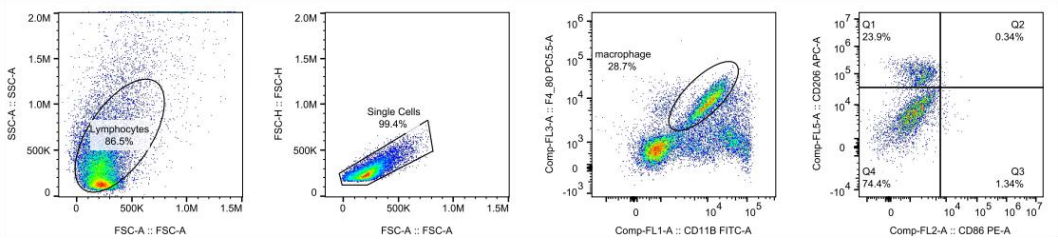
Fig2 Statistical chart of macrophage ratio in mouse lung tissue



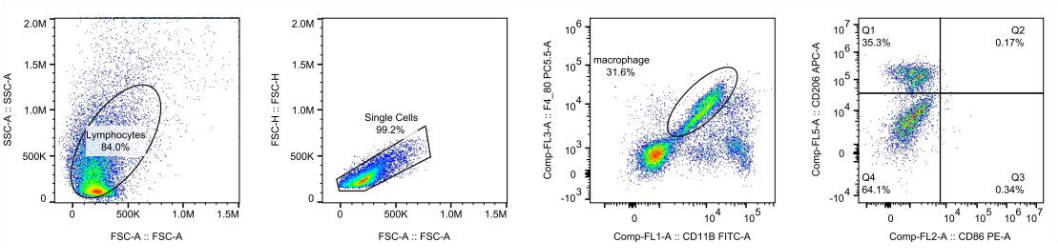
Uninfected group（a）



3d（b）



5d（c）



7d（d）

Fig3 Flow cytometry chart of lung tissue macrophages

Note: In Figures 2 and 3, 0d represents the uninfected group;

Figure 3 shows flow cytometry images of lung tissue from different groups of mice. CD11B+F4/80+represents macrophages, CD206+represents M2 macrophages, and CD86+represents M1 macrophages;

4 | Discussion

S.aureus infection still plagues clinical workers till this day, and its powerful virulence factors, including toxins, immune evasion factors, and invasive enzymes, pose a significant threat to the body. The lung infection caused by it can lead to symptoms such as high fever, chest pain, purulent sputum, lung consolidation, pneumothorax, and pyopneumothorax while some infected patients' other organ systems may also be damaged. By this time, early diagnosis and treatment is particularly important. The gold standard for clinical diagnosis of S. aureus is the cultivation of bacteria from blood samples of patients, but this method costs a long testing time and shows an

unfavorable positive rate. At present, researchers have used digital PCR to detect gene fragments related to S. aureus in blood samples, which significantly reduces the time required for testing[9]. For patients with a clear diagnosis of infection, the early and targeted use of antibiotics can help them recover as soon as possible. Along with the use of antibiotics, the issue of drug-resistant bacteria inevitably comes into the picture. Bacterial resistance has multiple mechanisms, such as changing their own drug targets and secreting enzymes to destroy drugs, etc. [10]. As the most notorious drug-resistant bacterium, MRSA makes our treatment not only limited to antibiotics. The immune links related to its infection are equally important for treatment. Different from other common pneumonia pathogens, S. aureus has a very complex immune evasion mechanism. Its own abundant immune evasion proteins can help it evade the recognition and phagocytosis of immune cells, interfere with the host's complement reaction, and resist antibacterial substances, etc. [11]. This is also one of the important reasons for the serious clinical harm after infection. And the powerful drug resistance mechanism of MRSA on this basis makes it impossible for us to rely solely on antibiotics for long-term treatment. Although new methods to combat MRSA are constantly being studied at present, most of them still remain at the experimental stage. Therefore, the immune exploration of infection is also particularly important.

As one of the immune barriers of the body, macrophages are usually believed to polarize towards M1 in the early stage of infection to help the body fight against invading pathogenic microorganisms, and then polarize towards M2 to repair the damage suffered from infection, such as in the model of Brucella infection[12]. However, not all microbial infections are the same. In the model of Chlamydia trachomatis respiratory infection, M1 macrophages always dominate[13].In this experiment, the macrophages in the lungs of the infected group significantly increased and polarized towards M2. The increased macrophages were recruited from other parts of the body through blood, but this didn't improve the condition. The inflammation in the lungs of the mice got worse and gradually led to their death. The body seemed to have abandoned the positive confrontation with MRSA. When S. aureus invades human or mouse bodies, it binds to receptors on the surface of macrophages, especially TLR2 receptors, guiding the aggregation of macrophages at the site of infection, resulting in a significant increase in macrophages at the site of infection. The FoxO1 transcription factor in macrophages is involved in regulating energy metabolism, inducing cellular responses, tumor proliferation, and many other reactions. In addition, it has been confirmed to play an important role in macrophage polarization, and the polarization of macrophages towards M1 in mice with this protein gene knocked out is significantly inhibited[14]. While the expression of FoxO1 protein is negatively regulated by the TLR2 receptor mediated signaling pathway. This may be one of the reasons why the polarization of mouse lung macrophages towards M1 macrophages is inhibited after MRSA infection, making the process of MRSA respiratory infection present a state similar to wound healing at the microscopic level, which is the high expression of M2 macrophages. However, due to the presence of pathogens, it hinders the progress of healing. Therefore, by clearing the pathogens, the tissue can be cured. For example, a large number of M2 macrophages are also detected in Mycobacterium tuberculosis granulomatous tissue, especially in patients with active Mycobacterium tuberculosis infection[15]. In clinical practice, we often use anti tuberculosis drugs to eliminate Mycobacterium tuberculosis and treat pulmonary tuberculosis. In the treatment of MRSA, vancomycin is used to eliminate the pathogen. In addition, in the infection

model of Mycobacterium tuberculosis, M1 macrophages are induced to produce NO and other active substances to affect the survival of Mycobacterium tuberculosis. In the surface wounds caused by MRSA infection, NO has been proven to have a good killing effect on MRSA. So whether it is possible to achieve the same goal by artificially stimulating macrophages to polarize towards M1, it remains to be experimentally verified. This experiment only preliminarily investigates the overall polarization of macrophages in lung tissues, which can be divided into interstitial macrophages and alveolar macrophages. Whether there is a difference between these two during the entire inflammatory process has not been verified. In addition, due to limitations in model animals, we are unable to analyze macrophages in the peripheral blood of mice to see if this polarization difference is limited to the lungs.

In summary, we have constructed a simple pneumonia model after MRSA respiratory infection and found that after infection with methicillin-resistant S. aureus in lungs, a large number of macrophages infiltrated and polarized towards M2 in lung tissues. Further research is needed to investigate the differences in polarization towards M1 macrophages and M2 macrophages, as well as the impact of M1 macrophages on the progression of inflammation.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material. Further inquiries can be directed to the corresponding authors.

Conflict of interest

The authors declared no conflict of interest.

References

[1] Cheung G Y C, Bae J S, Otto M. Pathogenicity and virulence of Staphylococcus aureus[J]. Virulence, 2021, 12(1): 547-569.

[2] Peacock S J, Paterson G K. Mechanisms of methicillin resistance in Staphylococcus aureus[J]. Annual review of biochemistry, 2015, 84: 577-601.

[3] Wielders C L C, Fluit A C, Brisse S, et al. mecA gene is widely disseminated in Staphylococcus aureus population[J]. Journal of clinical microbiology, 2002, 40(11): 3970-3975.

[4] Bassetti M, Labate L, Melchio M, et al. Current pharmacotherapy for methicillin-resistant Staphylococcus aureus (MRSA) pneumonia[J]. Expert opinion on pharmacotherapy, 2022, 23(3): 361-375.

[5] Cong Y, Yang S, Rao X. Vancomycin resistant Staphylococcus aureus infections: A review of case updating and clinical features[J]. Journal of advanced research, 2020, 21: 169-176.

[6] Italiani P, Boraschi D. From monocytes to M1/M2 macrophages: phenotypical vs. functional differentiation[J]. Frontiers in immunology, 2014, 5: 514.

[7] Jablonski K A, Amici S A, Webb L M, et al. Novel markers to delineate murine M1 and M2 macrophages[J]. PloS one, 2015, 10(12): e0145342.

[8] Ryszer T. Understanding the Mysterious M2 Macrophage through Activation Markers and Effector Mechanisms[J]. 2015.

[9] Ogihara S，Saito R，Sawabe E，et al. Molecular typing of methicillin-resistant Staphylococcus aureus：Comparison of PCR-based open reading frame typing，multilocus sequence typing，and staphylococcus protein a gene typing［J］. J Infect Chemother，2018，24（4）：312-314.

[10] Boyle-Vavra S, Daum R S. Molecular strategies of Staphylococcus aureus for resisting antibiotics[J]. Staphylococcus Genet. Physiol, 2016: 249-300.

[11] Flannagan R S, Heit B, Heinrichs D E. Antimicrobial mechanisms of macrophages and the immune evasion strategies of Staphylococcus aureus[J]. Pathogens, 2015, 4(4): 826-868.

[12] Xavier M N, Winter M G, Spees A M, et al. PPARγ-mediated increase in glucose availability sustains chronic Brucella abortus infection in alternatively activated macrophages[J]. Cell host & microbe, 2013, 14(2): 159-170.

[13] XU Y, NIU W, JING Y, et al. Chlamydia muridarum respiratory infection induces macrophage infiltration and polarization toward M1 phenotype[J]. Chinese Journal of Microbiology and Immunology, 2022: 194-201.

[14] Wang Y C, Ma H D, Yin X Y, et al. Forkhead box O1 regulates macrophage polarization following Staphylococcus aureus infection: experimental murine data and review of the literature[J]. Clinical reviews in allergy & immunology, 2016, 51(3): 353-369.

[15] Huang Z, Luo Q, Guo Y, et al. Mycobacterium tuberculosis-induced polarization of human macrophage orchestrates the formation and development of tuberculous granulomas in vitro[J]. PloS one, 2015, 10(6): e0129744.