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REVIEW ARTICLE

Research progress in immunological mechanisms of Cryptococcus

Ying Song[1], Yufang Qiu[1], Weiyou Liu[2], Xiaoliang Yuan[2*]

[1] *Gannan Medical University, Ganzhou 341000, Jiangxi Province, China.*

[2] *Department of Respiratory Medicine, The First Affiliated Hospital, Ganzhou 341000, Jiangxi Province, China. E-mail: yxlyyxs@126.com*

ABSTRACT

Whether infection of Cryptococcus causes disease in host or not depends on the virulence of the pathogen and the immune defense ability of the host. Cryptococcus neoformans (C. neoformans) mainly causes opportunistic infections in the immunocompromised or immunodeficient patients. In contrast, Cryptococcus gattii (C. gattii) mainly attacks the immunocompetent individuals. On the one hand, the host immune cells can eliminate the invasive Cryptococcus through a complex immune mechanism; on the other hand, Cryptococcus can evade the clearance of host immune cells by adopting various strategies (immune escape). This review mainly focuses on the pathogenic mechanism of Cryptococcus, and the host's immune defense mechanism against cryptococcal infection.

Keywords: Cryptococcus; Immune Mechanism; Macrophage; Dendritic Cells

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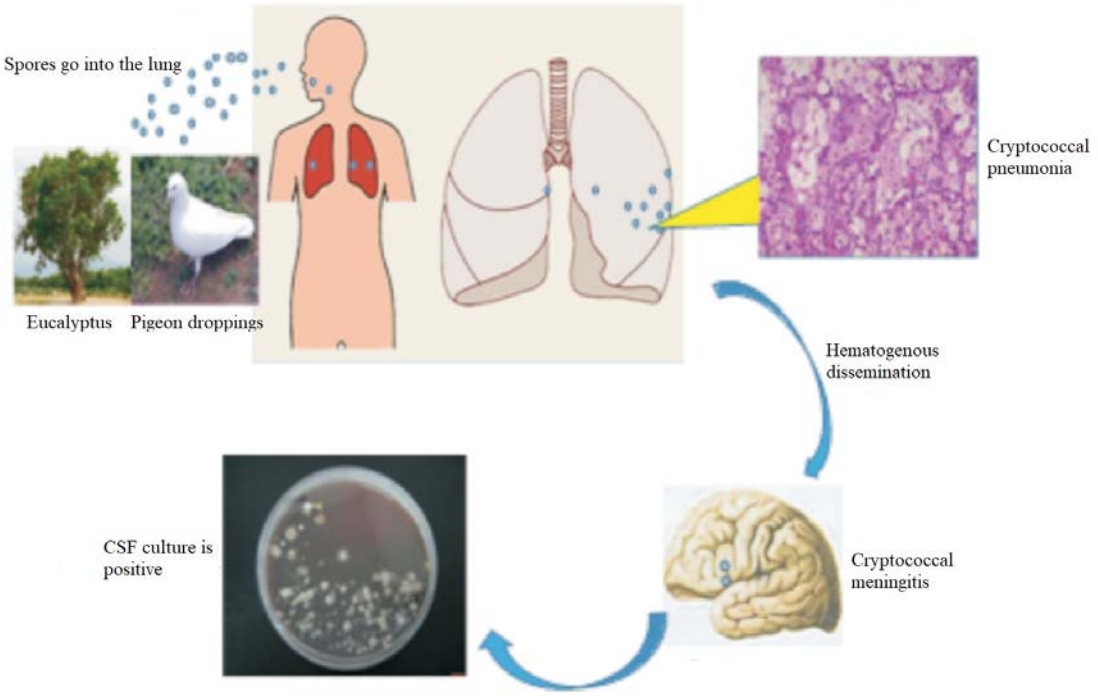
1. Introduction

Cryptococcus belongs to the subfamily of fungal basidiomycetes, including many species. There are mainly two kinds of conditional pathogens causing human opportunistic infection: Cryptococcus neoformans (C. neoformans) and Cryptococcus gattii (C. gattii). Cryptococcus neoformans is distributed in nature all over the world, mainly in soil and rotten vegetables, especially in pigeon droppings. C. gattii mainly exists in Eucalyptus, distributed in tropical and subtropical areas. In 1999, it broke out in temperate Colombia and spread to Washington, Oregon and California[1]. However, originally reported in the tropics, C. gattii infection is now diagnosed worldwide[2]. C. neoformans infection is the leading cause of death among AIDS patients worldwide. Especially in sub Saharan Africa, the incidence rate is the highest[3]. In addition to easily cause infections in HIV infection, C. neoformans also attacks other individuals with low immune function, such as hematopoietic malignancies, immunosuppressants after organ transplantation and patients with immune deficiency diseases. C. neoformans mainly affects individuals with normal immune function, but there are some special reports about C. neoformans infection in some immunocompetent patients and C. gattii infection in patients with immunodeficiency, such as those with HIV[4].

Cryptococcus is widely distributed in the air in the form of spores, inhaled into the lungs and deposited in the alveoli through the human respiratory tract. When the host's immune function is normal, most of the invasive C. neoformans are cleared by the host, so there are no obvious infection symptoms. However, when the immune function is

damaged or low, a small number of Cryptococcus colonized in the host cells multiply, causing cryptococcal pneumonia. It also spreads through the blood-brain barrier and invades the central nervous system, causing cryptococcal meningitis[5], which is characterized by pneumonia such as cough, pleurisy chest pain, fever and dyspnea, and a series of clinical symptoms of meningoencephalitis. Cryptococci can be cultured in cerebrospinal fluid (**Figure 1**). The main symptom of C. neoformans infection is meningoencephalitis, while C. gattii infection is more common in the lungs[6]. The study results of animal models also support the difference between the two kinds of pathogens on the main target organs: mice infected with C. neoformans die of central nervous system infection, while those infected with C. gattii die of lung infection[7]. It shows that the two species have different effects on their target organs, but its mechanism has not been fully clarified. At present, the research on regulating and enhancing hosts' defense mechanism through immune has attracted extensive attention.



Cryptococcus in the air is inhaled by the host in the form of spores and deposited in the alveoli. When the immune function of the body is low or damaged, cryptococci colonized in the host cells proliferate in large numbers, causing cryptococcal pneumonia, which spreads through the blood-brain barrier, invades the central nervous system, and causes cryptococcal meningitis. Therefore, cryptococcus is often cultured in the cerebrospinal fluid of patients with cryptococcal meningitis.

Figure 1. The main pathway of cryptococcal infection.

2. Cryptococcus is pathogenic

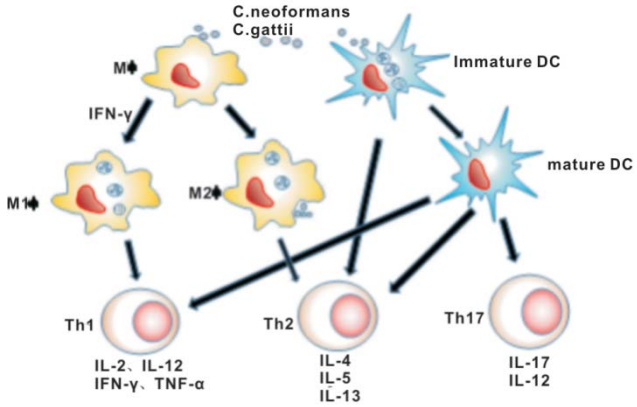
Cryptococcal capsule is an important virulence factor. Its main components are glucuronoxylan-nan (GXM), galactose xylose mannan (GalXM) and a small amount of mannose protein (MP), among which GXM accounts for more than 90% of polysaccharide components[8]. Cryptococcal virulence factors can interfere with the host protective immune response, including the defense of dendritic cells (DCS) and macrophages (Mφ) and antigen-presenting cells of the bone marrow lineage of monocyte precursors. In addition to producing specific enzymes and structures conducive to the survival of pathogens, the cell wall structure of Cryptococcus also actively regulates host specific signal transduction. This remolded structure leads to immune escape by shielding more immunogenic surface features[9]. Cryptococcus can evade clearance of host immune cells by adopting various strategies and successfully damage the defense mechanism of the host. GXM can not only adhere to the cell wall to form a capsule structure, but also secrete into the surrounding environment with a large amount (exo-GXM). The virulence and fungal load of mouse infection are related to the release of exo-GXM. During disseminated infection or intracranial infection, exo-GXM can prevent immune cells from infiltrating into the brain and inhibit inflammation[10].

3. Effect of phagocytes on Cryptococcus

After infecting the host, Cryptococcus interacts with different phagocytic effector cells[11]. Macrophages and dendritic cells (DC) play an important role in anti-Cryptococcus. Cryptococcus exists in the air in the form of spores, is inhaled into the alveoli through the respiratory tract and contacts with phagocytes. Phagocytes act as the first immune defense of the host to phagocytize, kill and invade pathogens and present antigens to activate T cells to mediate adaptive immune response. However, C. neoformans can replicate in phagocytes and escape to the extracellular environment through non lytic exocytosis, avoiding the clearance of phagocytes[12]. The research shows that during the growth, Cryptococcus removes maturation markers Rab5 and Rab11 of phagosome, and inhibit the maturation of phagocyte lysosome, and the acidification, calcium channel and enzyme activity of phagosome is blocked, which makes Cryptococcus proliferate in cells.

3.1 Macrophage

Macrophages can become the hub of innate and adaptive immunity after phagocytosing Cryptococcus. However, Cryptococcus can survive and proliferate in the phagocytosis of these infected host cells. Cryptococcus can escape host immunity by cleaving macrophages, but the mechanism of cleavage is not clear, which may be due to the rupture of host cell membrane caused by a large number of intracellular Cryptococcus replication. This shows that Cryptococcus can take macrophages as a protective area in the host. Chrissy M reviewed the interaction between Cryptococcus and phagocytes in detail[14]: macrophages can efficiently phagocytize Cryptococcus, but Cryptococcus has a variety of virulence factors to resist phagocytosis or enhance its reproductive ability in phagocytosis. However, a recent study[15] has explored the mechanism of nonspecific uptake of Cryptococcus by macrophages. Macrophages ingest Cryptococcus through mannose receptor (MR), ingest C. neoformans through dectin-1 and dectin-2, and ingest C. gattii through dectin-1, which proves that macrophages' important role of resisting Cryptococcus. Macrophages, as antigen-presenting cells (APC), promote T lymphocyte activation, induce Th1-like reaction and eliminate fungi. M1 type (classically activated) macrophages mediate Th1 response (mainly IFN-γ mediation), leading to the up regulation of reactive oxygen mediators, reactive nitrogen substances, proteases and lipid mediators, so that macrophages can effectively kill pathogens. Th1 stimulation can also increase the presentation of major histocompatibility complexes (MHC-I or MHC-II) and mediate adaptive immunity by reducing the activity of phagocyte hydrolases. M2 type (selectively activated) macrophages mediate Th2 response, help to inhibit and regulate inflammatory response, and play a role in the healing process, but have no killing effect on Cryptococcus[16] (**Figure 2**).



After Cryptococcus is recognized and phagocytosed by APC (Mφ and DC), M1 macrophages mediate Th1 response, and M2 macrophages mediate Th2 response; after immature dendritic cells (DC) phagocytose Cryptococcus, , the expression of maturation markers CD80, CD86 and MH CII on cell surface increases, which mediate the differentiation of CD^{4+} T into Th1, Th2 and Th17 cells. They produce different inflammatory cytokines and inhibitory cytokines. Immature DC can also mediate Th2 reaction.

Figure 2. Effect of phagocytes on Cryptococcus.

3.2 Dendritic cell

As full-time APCs, dendritic cells mainly regulate and activate the adaptive immune system according to the polymorphism of antigen, and produce a specific immune response to infection. After Cryptococcus invades the lung, DC preliminarily processes Cryptococcus antigen through the endosomal/lysosomal pathway, presents it with MHC-class II molecules, and kills Cryptococcus through oxygen dependent and oxygen independent mechanisms[17]. It was found[18] that within 2 hours

after intranasal inoculation of C. neoformans in mice, C. neoformans could be internalized by lung DC, lung macrophages and neutrophils; after 7 days of infection, the expression of maturation markers CD80, CD86 and MHC-II increased. It shows that DC gradually develops into mature DC after phagocytosis of C. neoformans, and can present C. neoformans antigen to specific T cells to activate T cells. Mature DC can effectively present antigen, start T lymphocytes and mediate Th1 and Th17 immune response, while immature DC can induce immune tolerance and mediate Th2 non protective immune response. Wozniak KL[19] clarified the process of DC recognizing, processing Cryptococcus and mediating immune response to Cryptococcus. It shows that DC cells play an important role in both innate and adaptive immune defense against cryptococcosis (**Figure 2**).

DC recognizes Cryptococcus presentation antigen and mainly stimulates T cell pathway. Although alveolar macrophages can also activate T cells through cryptococcal antigen presentation, the T cell effect stimulated by DC is more effective. The experimental results showed that[20]: cryptococcal antigen stimulated bone marrow dendritic cells (BMDC) to induce the release of protective immune factors IL-12/23p40, but did not release these protective factors after stimulating bone marrow macrophages. The possible reason for this difference is that after cryptococcal antigen stimulation, BMDC up regulates MHC-II and CD86, while bone marrow macrophages down regulate MHC-II and CD86. DC has many subtypes according to different sources, and different subtypes have different characteristics in anti-cryptococcal infection. Plasma cell like DCs phagocytize C. neoformans and limit its growth through dectin-3 and reactive oxygen species dependent mechanisms[21]. The protective immune response against cryptococcal antigen is mediated by $CD11b^+$ DC and Langerhans cells[22]. $CD11b^+$ DC can also mediate non protective Th2 response[23]. Recent studies have found that Cryptococcus can use the collagen structure of macrophage receptor to promote the accumulation of $CD11b^+$ DC and change the Th1/Th2 balance, which is conducive to the reproduction and spread of fungi. Monocyte derived DCs enhance Th1 response after respiratory tract infected with C. neoformans.

3.3 Effect of T cells on Cryptococcus

Patients suffered from C. neoformans with AIDS are closely related to T cell defects. T cells are necessary for adaptive immune response. In human body, $CD4^+$ T cell defect is the main factor inducing cryptococcosis, in which the count of $CD4^+$ T cells is less than $100 \cdot \mu \cdot L^{-1}$, indicating an increased risk of HIV related cryptococcosis[25]. T lymphocytes that participate the response of the host to C. neoformans include $CD4^+$ T cells, $CD8^+$ T cells and natural killer T (NKT) cells. $CD4^+$ T cells, $CD8^+$ T cells and NK cells can directly bind to Cryptococcus and act in a way of inhibiting fungi. Recently, an auxiliary T cell (CD^{4+} Fox P3 Treg) was found to inhibit Th2 response in anti-Cryptococcus[26]. Activated $CD4^+$ T cells can activate and proliferate B cells, macrophages and $CD8^+$ T cells to produce antibodies. $CD8^+$ T cells play an important role in the host immune response to C. neoformans[27]. Both $CD4^+$ T cells and $CD8^+$ T cells produce pro-inflammatory cytokines against Cryptococcus. $CD8^+$ T cells contact C. neoformans cells directly and release granulysin to kill C. neoformans.

$CD4^+$ T cells are the key to regulating the type of immune response. Naive $CD4^+$ T cells are activated and differentiated into different subsets of Th1, Th2 and Th17 to produce cytokines. Th1 type regulates the host to induce cellular immune response and produce cytokines IL-2, IL-12, IFN-γ and TNF-α, having a protective effect against Cryptococcus[28]. Th17 is necessary for vaccine mediated protection of mice against C. neoformans[29], and mainly secretes cytokines IL-17 and IL-22. Th2 reaction produces cytokines such as IL-4, IL-5 and IL-13, which has a non-protective effect on Cryptococcus infection. In HIV infection, cytokines change from Th1 to Th2, and the host immune environment becomes more conducive to cryptococcal infection and diffusion (**Figure 2**).

4. Conclusions

The diseases and mortality caused by Cryptococcus infection in the world are very high every

year. Because Cryptococcus has unique virulence factors, such as capsular polysaccharide, which plays an important role in resisting the immune response of the body and can escape the clearance of host cells. At present, although there is continuous progress in the study of the pathogenic and immunological mechanism of cryptococcosis, it is still not enough to effectively control the epidemic of cryptococcosis. C. neoformans adapts to the intracellular environment and resists the immune response of the host through a variety of strategies. For example, C. neoformans colonizes macrophages, symbiotically proliferates and escapes to the extracellular environment, causing disease dissemination. Therefore, future research will need to pay attention to the parasitism capacity of Cryptococcus in host cells and related immune mechanisms.

The body's protective immunity against Cryptococcus requires T cell response, which produces the key protective inflammatory factor TNF-α, IL-12 and IFN-γ. These responses are triggered by classical DC activation. DC plays an important role in phagocytosis and killing Cryptococcus. Studies have shown that TLR4 and TLR2 on the surface of DC can recognize the capsule component GXM of Cryptococcus[30]. Therefore, an in-depth understanding of the interaction between DC and Cryptococcus will help to improve the immunotherapeutic effect of Cryptococcus infection in the future.

In the model of Cryptococcus infection in mouse lung, early inoculation of IL-12 can reduce the load of Cryptococcus in lung and inhibit its diffusion to brain, and the therapeutic effect of IL-12 is related to the production of high concentration of IFN in lung-γ[31]. Immunocompromised patients were given recombinant IFN-γ1b can promote the killing of Cryptococcus in cerebrospinal fluid and increase the body's drug resistance[32]. If we want to improve the immune efficacy of cryptococcal infection treatment, we should deeply understand the signal transduction pathway involved in cryptococcal pathogenesis.

Conflict of interest

The authors declare no potential conflicts of interest.

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