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Understanding the early host immune response against *Mycobacterium tuberculosis*

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Abstract

Generation of immune response is a crucial activity of host defense against any microbial attack. When facultative organism Mycobacterium tuberculosis (MTB) invades its host, various pathways are activated in the host to mount immune responses against invading pathogen for nullifying its actions. During this host-pathogen interaction, interplay of complex network of cytokines and chemokines, initiation of phagocytosis, and formation of granuloma play an important role in containing MTB infections at host side. Simultaneously, MTB also evolves a plethora of specialized mechanisms to evade the host's killing cascades on other side, and during this bilateral cross-talk, many mycobacterial products play crucial role in survival of MTB inside the host. Hence, a better understanding of these phenomena is necessary not only for getting clear picture of pathogenesis of MTB, but also for developing effective, preventive, and therapeutic modalities against the pathogen. With some suggestions on future work, an insight into diversity of immune response of host against MTB was provided in the present review.

Key words: *Mycobacterium tuberculosis, host, immune response, immune evasion.*

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Introduction

In the evolutionary niche of microbial world, tubercle bacilli (originally named *Bacterium tuberculosis*) further renamed as *Mycobacterium tuberculosis* (MTB), a landmark discovery of noted microbiologist Heinrich Hermann Robert Koch stands out with its impact on actual history of mankind. Having the close intimacy with genera *Corynebacterium*, *Rhodococcus*, and *Nocardia*, *Mycobacterium tuberculosis* is a genuine member of "single species containing" genus "*Mycobacterium*", which has been placed in the *Mycobacteriaceae* family [1]. Characteristically, it is an aerobic, slow growing, non-motile, non-spore forming, and acid-fast bacilli (AFB) with facultative nature.

Mycobacterium tuberculosis is an etiological agent of tuberculosis (TB), a disease that ranks above AIDS in causing worldwide mortality and morbidity. In 2016, tuberculosis claimed 1.3 million lives in HIV-negative people, in addition to 374,000 lives in HIV-positive people. About 6.3 million people got new MTB infections across the globe. One third of population of the world is believed to be latently infected with MTB, of which about 5-15% will develop active disease with favorable conditions [2]. Although, best therapeutic modalities are available, the tuberculosis remains a major challenge around the world. The emergence of various drug-resistant forms of MTB, poor adherence to treatment protocol, poor hygienic and nutritional status, smoking, and alcohol consumption are the possible factors responsible for this situation [3, 4]. The administration of certain drugs and microbiological product has also been found as iatrogenic cause of TB [5-7].

Since effective control of this disease is of prime importance, there is a necessity to have better understanding of the complex biology of MTB-host interactions, particularly of host immune response. Considering this requirement, in the present review, various aspects of host immune response against MTB were discussed in great details.

The route of entry of *Mycobacterium tuberculosis*

The mechanism of pathogenesis of MTB is very complex and partially understood. The initiation of pathogenesis possibly occurs with the entry of MTB into the host body through three possible routes such as 1) inhalation of contagious droplet nuclei harboring MTB into the respiratory tract; 2) gastrointestinal; 3) cutaneous [8]. Of these, respiratory tract is the widely acclaimed gateway for the introduction of MTB inside the host and subsequent progression of the disease. The number of individuals harboring MTB in general population varies because the source of infection and degree of exposure are mainly unknown. However, it is assumed that about 90% individuals of in-

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fected population have MTB infections and survive lifetime due to their strong immunity [9].

Cross-talk between *Mycobacterium tuberculosis* and macrophage: inside story

After inhaling by host, only a small fraction (10%) of MTB reaches the bronchioles and alveoli of respiratory tract [10], where they interacts with variety of host cell receptors including Fc receptors (FcR), complement receptors (CR), surfactant protein receptors, macrophage mannose receptor (MMR), dectin-1, dectin-2, DC-SIGN, Nods, and Toll-like receptors (TLRs), particularly TLR2/4 of macrophages [11]. Once MTB is engulfed by macrophages, the process of phagocytosis is initiated to kill the tubercle bacilli residing in phagosomes. This includes the fusion of MTB-containing phagosomes with lysosomes that resulted in development of phagolysosomes [12]. However, on other side, for evading the host immune responses particularly killing mechanisms of lysosome, MTB tries to stop the maturation of MTB-containing phagosomes into phagolysosomes [13], and maintain a conducive environment for its survival in phagosome. In this process, exclusion of vacuolar H^+-ATPases from MTB-containing phagosomes plays a vital role in assisting the pathogen to survive within the acidic environment of phagosomes [14]. Apart from this, tethering [15] and fusion machinery involved in trafficking of intracellular vesicles [16], including SNARE proteins (soluble N-ethylmaleimide-sensitive factor-attachment protein receptor) and small GTP-binding proteins of the Rab family such as Rab5 and Rab7 [17], are crucial for the biogenesis of phagolysosome [18]. Surprisingly, a 43 kD tryptophan-aspartic acid repeat actin-binding protein of the host, i.e. TACO (tryptophan aspartate rich coat protein, also known as coronin-1) takes part in the intracellular survival of MTB in phagosomes [19].

Granuloma formation: cardinal sign of host immune response

The formation of granuloma is thought to be a cardinal feature of early host immune response against MTB. Therefore, a great deal of effort has been made over the last years across the world to understand the mechanism of granuloma formation and its need in defense against MTB. A series of studies suggests that by forming the granuloma, host attempts to contain MTB infection in macrophage and limits its further dissemination [20]. However, the formation of granuloma itself is a very complex mechanism, and till date not fully understood. Although, genesis of granuloma is assumed to be due to active involvement of both innate and acquired immune responses including variety of immune cells, cytokines/chemokines, cell-adhesion molecules (CAMs), and various signaling cascades [21-24]. Not only the host immune response, bacterial component like trehalose 6,6'- dimycolate (TDM) (cord factor) is also required for initiating the formation of granuloma [25].

Further, for elucidating the formation of granuloma and their importance in the pathogenesis of MTB, several hypotheses have been proposed. A hypothesis postulated by Dannenberg, which was based on findings of their work on rabbit model suggested that macrophages containing MTB send intracellular signals to neighboring immune cells for their deployment at the site of infection, thus, resulting in organization of cellular structure around infectious macrophages, which is known as granuloma [26, 27]. Another hypothesis suggested that just after MTB infection, neutrophils migrate first towards the site of infection followed by aggregation of monocytes, which take 2 to 3 days in differentiating of macrophages, and a well-structured granuloma is developed within 5 to 7 day of infection [28].

Studies were also carried out to provide insight into formation of granuloma in human cases. The classical type of human granuloma is made up of a central necrotic zone and an outer layer of leukocytes. The central necrotic area, which serves as source of nutrition for intracellular MTB is further surrounded by foamy macrophages, epithelioid cells, and Langhans giant cells (which are generated by epithelioid cells fusion), while the outer thick leukocytic wall preventing the dissemination of MTB is composed of a mixture of immune cells including $CD4^+$ and $CD8^+$ T cells [29]. When we compare the architecture of human and mice granuloma, we can find that the human granuloma structurally differs from mice granuloma, but the cells involved in its formation are almost similar in both the cases. Moreover, the mice granuloma, which lacks Langerhan's giant cells is a collection of loosely gathered activated and epithelioid macrophages as well as other immune cells including lymphocytes. Despite these architectural differences in human and mice granuloma, the function of granuloma is almost similar in both the cases [30, 31].

In the development of granuloma, the role of lymphocytes was found to be step guiding. Experimental evidences suggest that initiation of formation of granuloma primarily needs activation of T lymphocytes [32]. Once MTB is engulfed by dendritic cells in the lung [33], antigen processing is taken place. Just after, carrier dendritic cells are migrated to the draining mediastinal lymph nodes, where they interact with T lymphocytes. After activation by dendritic cells, T lymphocytes mediate protective immune response against MTB [34, 35]. Contrary to these observations, a study on MTB-infected mice suggested that for mediating the protective immune response against chronic infection of MTB, the presence of granuloma is necessary in addition to T lymphocytes [36]. The upregulation of members of cell adhesion molecules (CAMs) family on both leucocytes and endothelial cells further mediates the deployment of mononuclear phagocytes and activated T lymphocytes to the site of infection, leading to extravasation of these cells into the lung [20]. When we look back at the background of granuloma

Table 1. Mediators of granuloma formation/maintenance

Mediators	Produced by	Major role
Cytokines		
IFN-γ	CD4+ and CD8+ T cells, NK cells	Macrophage activation
TNF-α	CD4+ T cells, macrophages	Macrophage activation, chemokine induction, granuloma maintenance
IL-1	Macrophages, dendritic cells, monocytes	Regulation of interferon (IFN) functions, recruitment of phagocytic cells
IL-6	Monocytes, fibroblasts, T cells, B cells	Granuloma maintenance
Il-10	Th2 cells, Th1, and Th17 cells, macrophages, dendritic cells, myeloid derived suppressor cells, B cells, neutrophils, Treg cells	Macrophage deactivation
IL-12	Dendritic cells, macrophages, B cells	Early T cell activation and polarization, T cells recruitment in developing granuloma
IL-13	Th2 cells, CD8+ T cells, NK cells, granulocytes (e.g. mast cells, eosinophils, basophils)	Necrotic granuloma formation
IL-17	CD+T cells (Th17), γδ T cells	Induction of chemokines CXCL9-11, mediating recruitment of T cells in granuloma, neutrophil recruitment, macrophage activation
IL-18	Macrophages	Neutrophil/monocyte accumulation, induction of IFN-γ by T cells
IL-23	Dendritic cells, macrophages	Required for IL-17 and IL-22 production
IL-27	Macrophages, dendritic cells	Limiting migration of T cells towards site of infection
TGF-β1	Lymphocytes, macrophages, monocytes, dendritic cells	Formation of fibrous capsule around granuloma, liquefactive necrosis, impairment of T cells functions
Chemokines		
CXCL8	Alveolar macrophage, monocytes, alveolar epithelial cells, bronchial epithelial cells	Recruitment of neutrophil, T lymphocytes, and basophils
CCL2	Monocytes, alveolar macrophages, alveolar epithelial cells, bronchial epithelial cells	Recruitment of macrophages/monocytes, T cells, and other immune cells, polarization of näive T cells to Th2 cells
CCL3/4/5	Alveolar macrophages, dendritic cells, bronchial epithelial cells	Recruitment of macrophages/monocytes, T cells, and other immune cells
CXCL9/10/11	Monocytes, alveolar epithelial cells, bronchial epithelial cells, dendritic cells, B cells	Recruitment of a variety of immune cells
CXCL13	Dendritic cells, pulmonary fibroblasts	B cells recruitments, granuloma associated follicular structure formation
CCL19/21	Stromal cells of secondary lymphoid organs	T cells recruitment, dendritic cells migration from lung to draining mediastinal lymph nodes

formation and containment of infection within granuloma, we found that a complex network of cytokines and chemokines plays a directing role in these cellular activities [37]. Table 1 summarizes the role of major effector molecules in granuloma formation and its maintenance.

Immune evasion by *Mycobacterium tuberculosis*

During the host-pathogen interaction, the host tries to kill the invading pathogen on the one hand, while on the other hand, the pathogen develops survival strategies to evade host defenses and as a result of this multidirectional interaction, many different kinds of effector molecules including pro-inflammatory cytokines IFN-γ and TNF-α are produced [38]. A wealth of experimental evidences generated from animal models suggests that both IFN-γ and TNF-α play a pivotal role in eliciting the anti-mycobacterial response against MTB, via inducible nitric oxide synthase (iNOS)-dependent mechanism. After activation of macrophages by these cytokines, a considerable amount of toxic reactive nitrogen intermediates (RNIs) and reactive oxygen intermediates (ROIs) are produced, which ultimately act against intracellular MTB residing

in macrophages and likely to kill the pathogen [39, 40]. To counteract, MTB induces the production of IL-10 for suppressing the activity of iNOS via MyD88-dependent mechanism [41]. Transforming growth factor β1 (TGF-β1) has also been reported to help the intracellular MTB in evading the host killing response by impairing the function of macrophages and T cells [42].

A number of mycobacterial proteins have been implicated in evading the host immune response [43-45]. Of these, small heat shock protein 16.3 (sHSP16.3) is a crucial protein of MTB that helps the bacteria not only in maintaining its long-term persistence [46], but also in continuing its growth in macrophages [47]. sHSP16.3 is also a good target for drug [48] and vaccine development [49, 50]. Recently, it was demonstrated that when MTB-infected macrophages are exposed to recombinant IFN-γ, the gene *hspX* encoding sHSP16.3 is up-regulated, whereas treatment of infected macrophage with recombinant IL-10 results in down-regulation of *hspX*. [51]. However, the mechanism facilitating this modulation is yet to be explored.

Conclusions

Although the host immune machinery against MTB is well explored in animal models, its various rate limiting steps are still not well understood in human cases. Future effort may be focused to study the underlying mechanism of formation of granuloma and its maintenance as well as to unravel the inside story of MTB-mediated evasion of host antimicrobial actions in human tuberculosis. The elucidation of role of small heat shock protein 16.3 (sHSP16.3) in conferring protection to MTB from hostile environment of macrophages may also give a new insight.

The author declares no conflict of interest.

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