



INTERACTION BETWEEN MYCOBACTERIA AND MACROPHAGES THROUGH THE COMPLEMENT SYSTEM

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ABSTRACT

Mycobacterium tuberculosis remains one of the most common causes of infectious disease morbidity worldwide. During the initial interaction with human host, *M. tuberculosis* uses macrophages as its primary host cell for survival and replication, which implies that this macrophage invasion by the mycobacteria is one of the critical aspects in establishing tuberculosis infection. Needless to say, *M. tuberculosis* has also identified numerous portals of entry for parasitizing macrophages, which include macrophage receptors that can recognize and bind to the bacilli. Besides this, the complement system, belonging to both innate and adaptive components of immune system and which is made up of more than 35 proteins, also contributes to the interaction between macrophages and mycobacteria. *M. tuberculosis* can bind to complement receptors *via* both complement-dependent and independent pathways. Complement component C3 identified as the major component in human serum is involved in enhancing the adherence and uptake of *M. tuberculosis* by mononuclear phagocytes. This review focuses mainly on the interaction of complement system and mycobacteria. Complement and adaptive immunity includes B-cell and T-cell mediated immunity, apoptosis, cytokine production, granuloma formation and immune regulation. The initial interaction on macrophage and mycobacteria will help delineate the contribution of this early host–pathogen interaction to the pathogenesis of tuberculosis.

KEY WORDS: Tuberculosis, Complement, Macrophage, Adaptive immunity, Apoptosis



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INTRODUCTION

Tuberculosis (TB) is a major global health problem. One third of the world's population is infected with *Mycobacterium tuberculosis*. It is estimated that 9 million new cases of TB and 1.4 million people died of TB. Geographically, the burden of TB is higher in Asia and Africa. India and China together account for almost 40% of the world's TB cases^{1,2}. *Mycobacterium* has the ability to enter a number of different cell types, but the macrophage is its primary host cell and survives and replicates inside these cells. They provide a first line of defence by phagocytosing microbes that enter the body. In addition to this key role in innate immunity, macrophages are also involved in the induction and regulation of adaptive immune response³. The complement system, a key component of innate immunity, is a group of serum and membrane-bound proteins and glycoproteins that participate in various aspects of the immune defence of the host⁴. Complement system in the presence of foreign molecules or immune complexes engages complex enzymatic cascades in which one complement component sequentially binds and activates another. Activation occurs through three distinct pathways: classical, alternative and lectin pathways, depending on the nature of the activating surface and the recognition molecules involved⁵. The interaction between the macrophage and mycobacteria is mediated by a variety of macrophage receptors. Mycobacteria can bind to the macrophage receptor through both complement-dependent and -independent pathways. In addition to complement receptors, there are other receptors involved in the adherence of *M. tuberculosis* to macrophages, such as mannose receptors, class A scavenger receptor, surfactant protein A receptor and CD14 receptor^{6,7}. The interaction of mycobacteria with macrophages represents an ideal opportunity for unraveling microbiological as well as host-cell biological mechanisms. In studying the interaction of mycobacteria with their host cells, it should be realized that mycobacteria have developed strategies to

circumvent the normal trafficking routes in macrophages in order to increase their chances of survival by manipulating the normal host cell biology. This review focuses mainly on the interaction of complement system and mycobacteria. Complement and adaptive immunity includes B-cell and T-cell mediated immunity, apoptosis, cytokine production, granuloma formation and immune regulation.

Overview of complement activation

The complement system consists of more than 35 soluble and cell bound proteins, 12 of which are directly involved in the complement pathways (Fig. 1). The complement proteins account for 5% of the serum globulin fraction. Most of these proteins circulate as zymogens, which are inactive until proteolytic cleavage. The complement proteins are synthesized mainly by hepatocytes; however, significant amounts are also produced by monocytes, macrophages and epithelial cells in the gastrointestinal and genitourinary tracts. Complement system is activated *via* three different pathways, classical, alternative and lectin pathway^{8,9}. Antigen-antibody complexes initiate the activation of the classical pathway, whereas the alternative and lectin pathways are activated in an antibody-independent fashion through interaction of complement components with specific carbohydrate groups and lipopolysaccharides present on the surface of foreign pathogens^{10,11}.

Activation of any of the three pathways leads to the formation of C3-convertases, which enzymatically cleave intact C3 molecules. C3 is cleaved to C3b, which binds covalently to targets. The three pathways differ considerably in their initial steps. Nevertheless, all pathways lead to the formation of C3b and C5b. C5b initiates the terminal pathway that generates the terminal complement complex C5b-C9 or Membrane attack complex (MAC). MAC can penetrate the target cell membrane and form pores that subsequently lyse the target cell (Table.1).

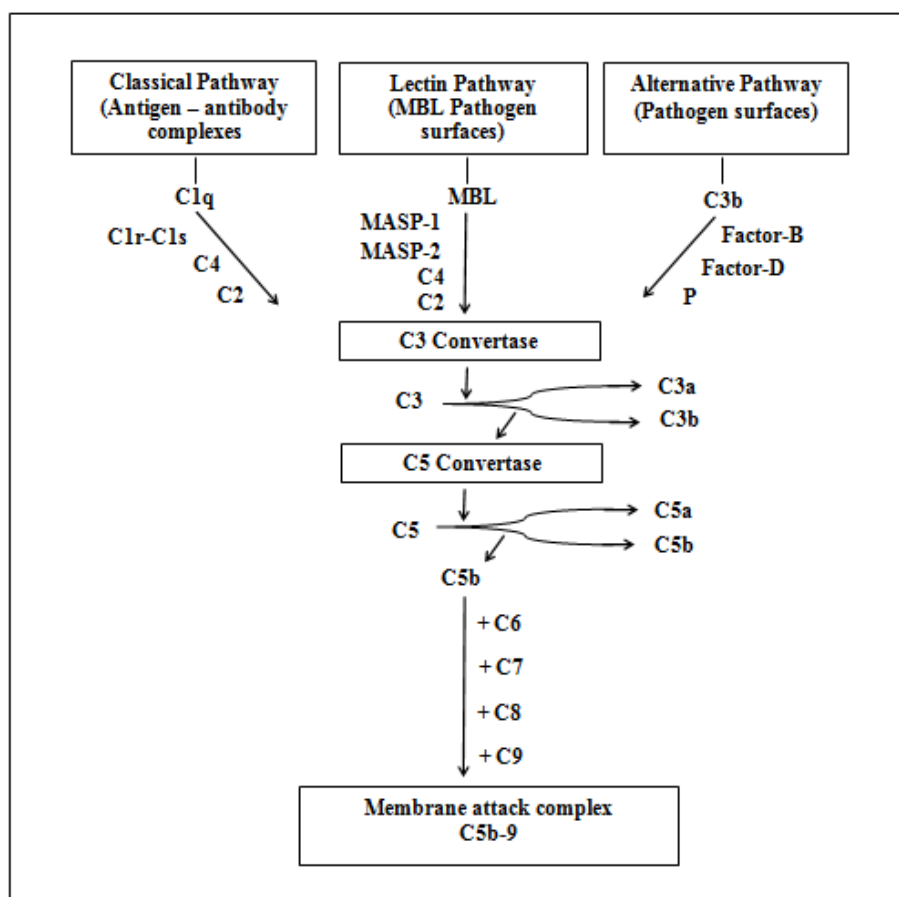


Figure 1

The three pathways of complement activation. A simplified schematic depicting the antibody-dependent classical complement pathway, the antibody-independent alternative pathway and lectin complement pathway. All three pathways merge at C3 and lead to the formation of the terminal complement complex (C5b-9).

The surface-attached C3b molecules are recognized by complement receptors on phagocytes, and small peptides (C3a and C5a) cleaved from C3 and C5 attract macrophages and neutrophils to the site¹². Taken together, complement can directly lyse the targets, mark them for phagocytosis and participate in generation of an inflammatory response.

Table 1
Complement cascades

	Complement cascades		
	Classical	Alternative	Lectin
Activators	Antigen-bound IgM and IgG	Pathogen surface molecules LAM and LPS	MBL–MASP protein complex
C3 convertase	C4b2b	C3bBb	C4ab2b
C5 convertase	C4b2b3b	C3bB3b	C4b2b3b
MAC	C5678 poly 9	C5678 poly 9	C5678 poly 9
Anaphylatoxins	C3a, C4a, and C5a	C3a, C5a	C3a, C4a, and C5a

(Abbreviations: LAM - Lipoarabinomannan, LPS - Lipopolysaccharides, MBL - Mannose binding lectin, MASP - MBL-associated serine protease, MAC - Membrane attack complex).

Interaction of complement with mycobacteria

The interaction between macrophage and mycobacteria is mediated by a variety of macrophage receptors (Table 2). The complement system is composed of a group of serum proteins and their corresponding receptors are located on the surface of phagocytes. *M. tuberculosis* can bind several types of receptors on the surface of phagocytes¹³. Complement receptor type 1 (CR1 or CD35) is a type 1 transmembrane glycoprotein of 220 kDa that contains 30 ScRs and is present on a wide variety of cells. CR1 is present in four different allotypes with different molecular masses of 160 kDa (A form), 190 kDa (B form), 220 kDa (C form) and 250 kDa (D form). It functions mainly as a receptor for C3b and C4b, but not C3bi. CR1 primarily functions in particle adherence rather than internalization¹⁴. CR1 possesses complement regulatory activity and can mediate phagocytosis of opsonized particles. CR1 protein carries the Knops blood group antigens and is the receptor for the major ligand involved in *M. tuberculosis* adhesion to macrophages¹⁵. Erythrocyte CR1 binds immune complexes (ICs) formed during *M. tuberculosis* invasion, facilitating their clearance by the host immune system.

Complement receptor type 2 (CR2 or CD21) is a 140 kDa membrane glycoprotein that binds to C3bi, C3dg and C3d fragment of C3. CR2 is implicated in the regulation of B-cell response and is involved in antibody response to T cell-dependent and -independent antigens¹⁶. The complement receptor type 3 (CR3 or CD11b/CD18) with a 170 kDa α chain and a 95 kDa β chain and the complement receptor type 4 (CR4 or CD11c/CD18) are adhesion molecules of leukocyte integrin family. CR3 and CR4 are both integrin heterodimers and have the same β chain, but different α chain. To date, CR3 has been the most widely studied receptor. CR3 mediates opsonization and phagocytosis of microorganisms. The two types of opsonic phagocytosis have been

defined depending on the receptor engaged¹⁷. Fc gamma receptor mediates type I phagocytosis of IgG-coated particles, CR3 mediates type II phagocytosis of complement-coated particles. Therefore, CR3 mediates type I phagocytosis under nonopsonic conditions and type II under opsonic conditions. CR3 mediates both types of phagocytosis depending on the ligand used.

CR3 and CR4 share considerable similarity at the amino acid levels and interact with the same ligand iC3b. Although CR3 binds to iC3b only, CR4 also interacts with C3d and C3dg; together with LFA-1, CR3 and CR4 form part of a super gene family of glycoproteins (integrins) that serve as receptors for adhesion molecules¹⁸. In contrast, CR4 is minimally involved in uptake of *M. tuberculosis* by monocytes compared with CR1 and CR3. The physiological role of CR4 is not clear, but its properties may be similar to those of CR3. During maturation of blood monocyte to alveolar macrophages, expression of CR3 decreases while that of CR4 increases¹⁹. *M. tuberculosis* can activate the alternative pathway of complement activation, resulting in opsonization with C3b and C3bi. Complement receptors interact with C3 deposited on *M. tuberculosis*, through the alternative complement pathway^{20,21}. Pathogenic mycobacteria (*M. avium*) uniquely recruit the complement fragment C2a to form a C3 convertase and generate opsonically active C3b in the absence of early activation of alternative or classical pathway²². In addition to complement receptors, there are other receptors involved in the adherence of *M. tuberculosis* to macrophages, such as mannose receptor, class A scavenger receptors, surfactant protein A receptor, Fc receptor and CD14^{23,24}. Hence, *M. tuberculosis* displays numerous and diverse ligands on its surface and is likely to engage multiple receptors simultaneously. The diverse array of receptors that could be utilized by mycobacteria to interact with and to enter host cells makes it unlikely that there is one 'preferred route'.

Table 2
Phagocyte receptors that bind *M. tuberculosis*

Molecule	Structure	Distribution	Ligands	Functions
CR1	Type 1 TM glycoprotein; monomer 4-allotypes, 160 kDa, 190 kDa, 220 kDa, and 250 kDa	Leukocytes	C3b and C4b	Binding with & opsonization by alternative and C2a pathways; cooperates with CR3 mediates phagocytosis
CR2	140 kDa membrane	Glycoprotein	B-cell, T-cell, and dendritic cell	B-cell activation, generation of immunological memory, and B-cell tolerance
CR3	$\beta 2$ integrin, $\alpha \beta$ heterodimer; CD11b/CD18, 170 kDa α chain and 95 kDa β chain	Myeloid and NK cells	C3bi, fB, ICAM, factor X, LPS, and β -glucans	Opsonic binding <i>via</i> C3bi and direct binding <i>via</i> d-glucans; absent O ₂ burst
CR4	$\beta 2$ integrin, CD11c/CD18 150 kDa α chain and 95 kDa β chain	Macrophages and Myeloid cells	C3bi	Opsonic binding <i>via</i> C3bi; supports O ₂ burst
C5aR	43-kDa	Neutrophils, macrophages and mast cell	C5a	Receptor for complement component C5aR, augment the humoral and cellular responses
C3aR	95 kDa	Monocytes, T-lymphocytes, and neutrophils	C3a, and C4a	Cell aggregation, adhesion, and immunoregulation
C1qR	CD93	Macrophages and neutrophils	C1q	Immune complex binding to phagocytes
CR1g	None	Macrophage	C3b and iC3b	Mediate phagocytosis

(Abbreviations: fB – factor B, ICAM-1 - Intercellular adhesion molecule, LPS – Lipopolysaccharide)

The complement system and adaptive immunity

Complement is a functional bridge between innate and adaptive immune responses that allows an integrated host defense mechanism. The complement system is an ancient mechanism for defense, and can be found in species that have been on Earth for the last 600 million years. However, the link between the complement system and adaptive immune responses through activation of distinct complement receptors on antigen presenting cells, B cells and T cells is a much more recent adaptation²⁵. Increasing evidence indicates that the complement system is essential for generating protective immune responses to mycobacterial infection²⁶, in addition to its clear role in both innate immunity and the initiation of adaptive immunity to a variety of other pathogens²⁷. Impaired acquired immune response has been observed in C3-depleted mice, suggesting that complement had an additional biological role and exemplified the

general immunological principle of the instruction by innate immunity of the acquired immune response²⁸. CR2, which binds C3 cleavage products, iC3b and C3dg, and is expressed on B cells as well as follicular dendritic cells (FDC), is instrumentally involved in the induction of a primary humoral response²⁹.

Complement and B cell immunity

The complement system of innate immunity is important in regulating humoral immunity largely through the complement receptor CR2, which forms a co-receptor on B cells during antigen-induced activation. The interaction between complement fragment C3d and CR2 is a key aspect in the link between innate and adaptive immunities³⁰. The co-engagement of CD21/CD19/CD81 receptor complex with the B cell antigen receptor enhances B cell responses by lowering the threshold for B cell activation. Further, CR1/CR2 plays important roles in retention of C3-loaded immune complexes in

the lymphoid compartment. Finally, complement factors of the classical pathway as well as CR1/CR2 play critical roles at distinct stages of B cell differentiation, some of which are critical to elimination of self-reactive B cells³¹. In addition to the prominent roles of C3 and C4 cleavage products, C5a has been described as a positive regulator of immune memory and naive B cell trafficking. Of note, naive, germinal center as well as memory B cells can produce C5 suggesting that B cells may serve as a local source of C5 in secondary lymphoid tissues³².

Complement and T cell immunity

In addition to regulatory effects on B cell immune responses, complement activation is also critical to the development of T cell immunity. The generation of an effective T cell response involves:

- (1) Activation of naive T cells in secondary lymphoid tissue.
- (2) Acquisition of effector function.
- (3) Migration to the site of antigen.

Activation of the classical pathway by natural IgM antibodies is critical for effective priming of CD8+ T cell responses³³. Complement also directly influences T cell activation and acquisition of effector functions. C1q binds T cells and C1q-opsonised immune complexes leading to activation of T cells *in vitro*, with increased secretion of TNF- α and IFN- γ , likely acting through the C1q collagen tail receptor. More likely, T cell CR2 functions as an adhesion molecule, increasing binding of C3 fragment opsonised B cells and other antigen presenting cells and thereby enhancing immune responses. C3 cleavage products can bind to CR3, CR4 as well as to Membrane cofactor protein (MCP or CD46) and Decay-accelerating factors (DAF or CD55), the activation of which may directly affect T cell priming or indirectly enhance the efficacy of antigen presentation by antigen presenting cells (APCs). Indeed, C3 deposition on peritoneal macrophages significantly enhanced the proliferation of antigen-specific T cells³⁴.

Complement and apoptosis

Apoptosis is a distinct form of cell death that is essential for the regulation of the immune system. The apoptotic cells are engulfed primarily by macrophages. Macrophages infected with mycobacteria undergo apoptosis, which is considered as an important innate defence mechanism that prevents spread of infection by sequestering pathogens within apoptotic bodies and protecting the surrounding tissue from their harmful effects³⁵. The mechanism by which macrophages recognize apoptotic cell is poorly understood. Macrophages bear an extensive repertoire of receptors, which could potentially mediate binding and engulfment of apoptotic cell³⁶. The multitude of receptors involved, the mechanisms underlying the recognition, engulfment and phagocytosis of apoptotic cells by macrophages are complex. Complement receptors are also required for efficient uptake or opsonized apoptotic cell, for which complement activation is required. Deficiencies in the early components of complement pathway would be predicted to result in impaired clearance of apoptotic cell³⁷.

Complement opsonins appear to have a role in the uptake of apoptotic cells, possibly by interaction with CR3 and/or CR4 on phagocytes³⁸. Serum factors were shown to increase by 3–10 fold the uptake of apoptotic cells by human macrophages³⁹. CR3 and CR4 are expected to have an impact on phagocytosis only after efficient opsonization of the apoptotic cells by iC3b⁴⁰. Tagging the surface of apoptotic cells with C3b and iC3b may not only promote efficient clearance of apoptotic cells but may also exert anti-inflammatory responses. The pro- and anti-inflammatory consequences of complement activation will depend upon the specific ligands that are involved and the co-receptors that are engaged. The complete molecular mechanisms underlying the induction of apoptosis by complement and the consequences of such regulation on adaptive immunity and infection in pulmonary tuberculosis remain elusive.

Complement and cytokines

Cytokines, including IFN- γ , TNF- α and IL-12, have been implicated in protective immune response to *M. tuberculosis*. The role of TNF- α during mycobacterial infection is complex, mediating both protection and tissue damage⁴¹. Infection of macrophages with *M. avium* in the presence of heat-inactivated serum resulted in significantly higher levels of TNF- α than its whole. The active serum counter parts play a suppressive role for complement in the induction of TNF- α ⁴². The complement-mediated opsonic interaction between mycobacterium and the infected macrophage via the CR3 and/or CR4 could be advantageous to the bacterium by avoidance of potentially harmful reactions, such as TNF- α production, which could lead to increased bacterial survival. Complement activation products and receptors are biologically relevant regulators of IL-12 production. Complement activation fragments appear to be able to both augment (C5a: C5aR) and suppress (C5a: C5aR; C3b: CD46; C3bi: CR3) monocyte/macrophage production of IL-12⁴³. C5 represents the most potent anaphylatoxin. In addition, C5a possesses immunoregulatory activities through the induction of cytokines (TNF- α , IL-1, IL-6, and IL-8) in human monocytes⁴⁴. The C5-deficient mice demonstrated an increased growth of bacteria following infection than sufficient mice⁴⁵. The relationships between complement and immunoregulation by cytokines deserve further investigation.

Complement and granuloma formation

Granuloma is a hallmark of protective immunopathological response of the host following infection with mycobacteria. The mechanisms underlying protective granuloma formation, which lead to the prevention of disseminated disease, have not been fully elucidated. Activated complement components are known to be chemotactic to macrophages and also activate them, which leads to the formation of granuloma. TNF- α is thought to be the major cytokine responsible for the formation and maintenance of mycobacterial antigen-induced granulomas⁴⁶. More importantly, TNF- α

is of high importance in triggering molecular mechanisms that provide protection against mycobacterial disease⁴⁷. Furthermore, TNF-deficient mice failed to form granulomas in response to mycobacterial infection, exhibiting delayed expression of chemokines and delayed recruitment of CD11b+ cells⁴⁸. C5 is another critical component in the granulomatous response. Its cleavage product, C5a, is a potent anaphylatoxin that recruits cells to inflammatory sites and induces the production of cytokine subsets⁴⁹. Complement C5-deficient A/J mice exhibit increased mortality and a markedly increased inflammatory response in the absence of granuloma formation in *M. tuberculosis* infection⁴⁵. Thus, C5 likely plays an important role in early maturation of the granulomatous response⁵⁰.

Complement system and immune regulation

Complement protein and complement receptors appear to be able to regulate the adaptive immune system. The classical pathway of complement activation is dependent on antibody-antigen engagement. The interaction between complement is in fact also necessary for maintaining the titer or repertoire of natural antibodies⁵¹. The complement system is known to play a critical role in the clearance of immune complexes, which activate complement through the classical pathway. Complement-mediated clearance of immune complex is another type of interaction between complement and adaptive immunity with serious pathophysiological implications⁵². Complement activation leads to the covalent attachment of C3b and C4b to the complex, thus facilitating their solubilization⁵³. The iC3b/C3dg/C3d receptor CR2 on B-cells forms a complex with CD19 and CD81, known as the B-cell co-receptor. Attachment of C3d to antigen, the result of complement opsonization of pathogen, renders such antigen more than 1000-fold more immunogenic for immunoglobulin production⁵⁴. Recognition of the ability of complement to regulate the responsiveness of the adaptive immune system has renewed the biological interest in complement.

CONCLUSION

Complement is one of the important effector and regulatory systems of innate as well as adaptive immunity to enhance host defence. Mycobacteria have developed a large number of mechanisms to enter macrophages. Thus complement opsonization of mycobacteria likely plays a critically important role in the first encounter of the microbe with the host. The interaction between mycobacteria and macrophage lineage lies at the centre of the host immune response and determines whether the survival, multiplication or cytolysis of these intracellular pathogens is achieved. Mycobacterial evasion of the host immune response includes inhibition of infected cell and resistance to the anti-microbial strategies of macrophages. This ability to survive within macrophage has been recognized for a long

time. However, the molecular mechanisms underlying the resistance towards degradation have been poorly understood. The initial interaction on macrophage and mycobacteria will help delineate the contribution of this early host–pathogen interaction to the pathogenesis of tuberculosis.

ACKNOWLEDGEMENT

S. Manivannan and V. Narayan Rao acknowledge the Council of Scientific and Industrial Research (CSIR), New Delhi, India, for providing the Senior Research Fellowship.

Conflict of interest:

Conflict of interest declared none.

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