Short Review

The Role of Toll-like Receptor 2 in Survival Strategies of *Mycobacterium tuberculosis* in Macrophage Phagosomes

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Abstract. *Mycobacterium tuberculosis (Mtb), an intracellular pathogen, is phagocytosed by alveolar macrophage but it is not digested; it survives, proliferates and establishes Mtb infections. The long-term survival mechanism of Mtb is not yet clear. The host's immune response to Mtb is mainly mediated by a Toll-like receptor 2 (TLR2) in macrophages. In the early stage of the immune response by macrophage activation through TLR2, the proliferation of Mtb is suppressed and there is a direct bactericidal effect or induction of apoptosis in infected macrophages. This indicates that TLR2 signaling functions as a defense system against Mtb infection. However, TLR2 signaling from Mtb also appears to be part of the Mtb strategy to escape immune responses by macrophages, such as has been observed when there has been a decrease in MHC-II expression or antigen-processing activity. TLR signaling is reported both to be and not be involved in the maturation of phagosomes, indicating the possibility of contrary influences. In this review, we summarize immune responses of macrophages through TLR2 in Mtb infection, its involvement in phagosome maturation and we describe survival strategies of Mtb through TLR2 signaling.*

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Survival of *Mycobacterium tuberculosis* (Mtb) in macrophages is caused by the inhibition of acidification of phagosomes by suppressing the association of vacuolar proton adenosine triphosphatase (V-ATPase) and the phagosome membrane, which is part of the process of phagosome maturation (1-4). Phagosomes containing Mtb are prevented from fusing with lysosomes and thus fail to form phagolysosomes; as a result, Mtb survives and proliferates in macrophages. However, the process by which Mtb inhibits phagosome maturation is not yet completely understood. Nor is it completely understood how the Mtb which are located in phagosomes maintain long-term survival.

Macrophages recognize Mtb through a toll-like receptor (TLR) 2 (5, 6). TLR expresses on macrophages as a pattern recognition receptor (PRR) and recognizes common structures of foreign substances (7). TLRs are type-I membrane proteins and contain an extracellular domain with leucine-rich repeats and a cytoplasmic portion with homology to the interleukin (IL)-1 receptor family (8). Mammalian TLRs comprise a large family consisting of at least 11 members. Each receptor recognizes different foreign substances (9-12). TLR2 recognizes various proteins or lipids such as 19 kDa lipoprotein of Mtb (13, 14), lipoarabino-mannann (15) and LprG lipoprotein (16). Signaling *via* TLR4 has been confirmed in components of HSP70 (17) and 38 kDa glycolipoprotein (18). In the early stage of immune responses by macrophages against an infection by Mtb, TLR2 signaling is reported to function in a way that allows Mtb to escape destruction by the immune system by inducing suppression of acquired immunity, while working as part of the phylactic system. In addition to this, TLRs, whose main function has been believed to be the recognition of foreign

substances and the production of inflammatory cytokines, are reported to influence the maturation of macrophage phagosomes and thus have contradictory functions. It appears that even though Mtb is recognized by macrophages through TLR2, Mtb may utilize TLR2 as part of its survival mechanism. This makes TLR2 a very intriguing molecule to study when trying to understand the survival mechanism of Mtb. Here, we survey findings on the responses of macrophages through TLR2 and the influence of TLR2 on the process of phagosome maturation in Mtb infection, and describe a new possibility for the role of TLR2 in the Mtb survival mechanism.

Defensive Responses by Macrophages to Mtb Infection by Signals Mediated by TLR2

Macrophages produce various cytokines or reactive oxygen in the early stage immune responses to Mtb infection. It has been shown that these immune responses are caused in a TLR2-dependent manner: the production of tumor necrosis factor (TNF) or IL-12p40 decreases in TLR2-knockout mice, whereas the production of these cytokines increases 4 hours or 24 hours after an infection by Mtb in normal mice (19). Moreover, in TLR2-knockout mice, it is reported that granuloma formation in the lung, which plays an important role in suppression of growth and confinement of Mtb in the lung, decreased. In addition, the number of Mtb bacteria in organs increased and the survival rate of host mice decreased accordingly (20, 21). Furthermore, as TLR signaling from Mtb is involved in the induction of inducible nitric oxide synthase (iNOS) and formation of NO products (13), macrophage activation through TLR is assumed to induce direct bacteriocidal activity against Mtb with reactive oxygen. In fact, activation of TLR2 by 19 kDa lipoprotein is indicated to increase NO production in RAW 264.7 cells and is reported to induce killing of intracellular Mtb 48 hours after infection in both NO-dependent and -independent pathways in mice and human monocyte macrophages and alveolar macrophages (22). These results indicate that recognition of Mtb mediated by TLR2 can induce activation of killing of Mtb.

Futhermore19 kDa lipoprotein, a membrane component of Mtb, is known to induce apoptosis in human monocyte macrophages (23). As this phenomenon is enhanced in CHO cells in which TLR2 is strongly expressed and reduced in THP-1 cells treated by anti-TLR2 antibody, it has been shown to be caused in a TLR2-dependent manner. Moreover, TLR2-dependent apoptosis in host macrophages decreases the survival rate of intracellular Mtb. The fact that induction of apoptosis in macrophages infected with Mtb induces activation of acquired immunity against Mtb indicates that this response supports immune defensive responses. The above findings suggest that TLR2 signaling functions as a defense system against Mtb thorough induction of inflammatory responses by macrophages.

Escape of Mtb from Immune Responses by Macrophages through TLR2 Signaling

It is reported that activation of TLR2 by Mtb promotes production of inflammatory cytokines and functions as a system to defend macrophages from infection. It has been reported that 19 kDa lipoprotein and LprG inhibit expression of MHC-II molecules and processing of antigen and presentation of MHC restrict antigen by macrophages in a TLR2-dependent manner (14, 16, 24). As inhibition of MHC-II antigen processing will cause a suppressive effect on cell-mediated immune responses such as suppression of induction of antigen production against Mtb, or delayed type allergic reaction, it is suggested to be a part of the Mtb mechanism of escaping detection. In addition, there is a report indicating that suppression of cellular immunity by the production of cytokine, such as IL-10 or IL-4, occurs because of priority signals through TLR2 and provides Mtb a mechanism for escaping the host defense system (25). As it may be difficult to consider that recognition of Mtb through TLR2 functions only as a phylactic system by macrophages, Mtb possibly possesses survival strategies using TLR2 in macrophages.

Involvement of TLR Signaling for Phagosome Maturation

As described above, TLR signaling by phagocytosis plays an important role in the inflammatory responses by macrophages. It has been reported that maturation of phagosomes containing some kinds of bacteria depends on stimulation by TLR. Although normal macrophage phagosome containing *Escherichia coli* or *Staphylococcus aureus* fused with lysosomes, MyD88$^{-/-}$ or TLR2×4$^{-/-}$ macrophages did not fuse with lysosomes (26). Thus, phagosome maturation may be controlled by TLR signaling. Conversely, it has been reported that stimulation of TLR4 during phagocytosis of apoptotic bodies delayed acquisition of lysosomal markers (27). Furthermore, Yates and Russell reported that phagosome maturation proceeded independently of stimulation of TLR signaling. They demonstrated that phagocytosis of mannose or IgG-coated beads was induced by phagosome acidification and/or fusion with lysosomes in TLR2$^{-/-}$ and/or TLR4$^{-/-}$ monocyte macrophages well as normal cells (28). In this way, although there is conflicting evidence regarding TLR signals in the maturation of phagosomes, the TLR signal was critically important for determining the fate of a phagosome after phagocytosis. Thus, modifying the TLR signal may provide the basis for developing novel therapies for this infection.

The Role of TLR2 in the Strategy of Mtb

Mtb, as an intracellular pathogen, is recognized and phagocytosed by macrophages and survives in phagosomes. The recognition of Mtb by macrophages is mediated by TLR2, such as 19 kDa lipoprotein and lipoarabinomannan. These findings have led us to hypothesize that the localization of Mtb and chronic survival in macrophages is assisted by TLR2. The survival strategy is benefited by the host defense mechanism through TLR2.

What is the role of TLR2 in the survival strategy by Mtb? As described above, infection of Mtb mediated by TLR2 induces inflammatory cytokines, suppresses proliferation of intracellular Mtb and prevents death of the host. In TLR2-knockout mice, there is acute infection with rapid bacterial proliferation resulting in the host's death. These phenomena indicate that TLR2 signaling functions for phylaxis of the host. However, as TLR2 becomes part of the survival strategy of Mtb, it also has a negative role. Immune responses through TLR2 are believed to induce extended survival conditions for Mtb in macrophages by the production of inflammatory cytokines that accumulate immune cells, which induces the formation of granulomas. Because of explosive proliferation, an acute infection of Mtb in TLR2-knockout mice results in the death of the host mice, leading to an unfavorable condition for the survival of Mtb. TLR2 signaling is believed to play an important role in the survival strategy of Mtb by allowing its continued existence in macrophages.

Perspective

In this review, we discuss the possibility that TLR2 signaling plays an important role in the survival strategy of Mtb. We think that Mtb has a survival strategy that uses inflammatory responses by macrophages after phagocytosis mediated by TLR2 to acquire an environment for long-term survival in phagosomes and to maintain extended residence in them. There have been no reports on whether or not the infection of Mtb through TLR2 affects maturation of Mtb phagosomes. Nevertheless, as Mtb modifies various functions of macrophages through TLR2, it may be possible to influence the maturation of phagosomes. We believe that additional studies on survival strategies of Mtb through TLR2 will lead to the development of novel therapies.

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