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The Role of Cholesterol Oxidase of Mycobacterium tuberculosis in the Down-Regulation of TLR2-Signaling Pathway in Human Macrophages during Infection Process

Authors : Michal Kielbik, Izabela Szulc-Kielbik, Anna Brzostek, Jaroslaw Dziadek, Magdalena Klink

Abstract : The goal of many research groups in the world is to find new components that are important for survival of mycobacteria in the host cells. Mycobacterium tuberculosis (Mtb) possesses a number of enzymes degrading cholesterol that are considered to be an important factor for its survival and persistence in host macrophages. One of them - cholesterol oxidase (ChoD), although not being essential for cholesterol degradation, is discussed as a virulence compound, however its involvement in macrophages' response to Mtb is still not sufficiently determined. The recognition of tubercle bacilli antigens by pathogen recognition receptors is crucial for the initiation of the host innate immune response. An important receptor that has been implicated in the recognition and/or uptake of Mtb is Toll-like receptor type 2 (TLR2). Engagement of TLR2 results in the activation and phosphorylation of intracellular signaling proteins including IRAK-1 and -4, TRAF-6, which in turn leads to the activation of target kinases and transcription factors responsible for bactericidal and pro-inflammatory response of macrophages. The aim of these studies was a detailed clarification of the role of Mtb cholesterol oxidase as a virulence factor affecting the TLR2 signaling pathway in human macrophages. As human macrophages the THP-1 differentiated cells were applied. The virulent wild-type Mtb strain (H37Rv), its mutant lacking a functional copy of gene encoding cholesterol oxidase (ΔchoD), as well as complimented strain (ΔchoD–choD) were used. We tested the impact of Mtb strains on the expression of TLR2-depended signaling proteins (mRNA level, cytosolic level and phosphorylation status). The cytokine and bactericidal response of THP-1 derived macrophages infected with Mtb strains in relation to TLR2 signaling pathway dependence was also determined. We found that during the 24-hours of infection process the wild-type and complemented Mtb significantly reduced the cytosolic level and phosphorylation status of IRAK-4 and TRAF-6 proteins in macrophages, that was not observed in the case of ΔchoD mutant. Decreasement of TLR2-dependent signaling proteins, induced by wild-type Mtb, was not dependent on the activity of proteasome. Blocking of TLR2 expression, before infection, effectively prevented the induced by wild-type strain reduction of cytosolic level and phosphorylation of IRAK-4. None of the strains affected the surface expression of TLR2. The mRNA level of IRAK-4 and TRAF-6 genes were significantly increased in macrophages 24 hours post-infection with either of tested strains. However, the impact of wild-type Mtb strain on both examined genes was significantly stronger than its ΔchoD mutant. We also found that wild-type strain stimulated macrophages to release high amount of immunosuppressive IL-10, accompanied by low amount of pro-inflammatory IL-8 and bactericidal nitric oxide in comparison to mutant lacking cholesterol oxidase. The influence of wild-type Mtb on this type of macrophages' response strongly dependent on fully active IRAK-1 and IRAK-4 signaling proteins. In conclusion, Mtb using cholesterol oxidase causes the over-activation of TLR2 signaling proteins leading to the reduction of their cytosolic level and activity resulting in the modulation of macrophages response to allow its intracellular survival. Supported by grant: 2014/15/B/NZ6/01565, National Science Center, Poland

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