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Article

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ROLE OF TOLL-LIKE RECEPTORS SENSING AND CONTROL OFMYCOBACTERIAL INFECTION

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Abstract

Microbial products including mycobacterial antigens are recognized by distinct Toll like receptors (TLR) resulting in activation of cells of the innate immune system. Several mycobacterial components such lipomannan have been identified with dual functions -activating antigen presenting cells via TLR2 and inhibiting TLR activation pathways in a TLR independent way. Ablation of TLR signalling as described for mice deficient for the common adaptor protein MyD88 revealed that TLRs are crucial for the activation of an innate immune response as MyD88 deficient mice are highly sensitive to infection with *Mycobacterium tuberculosis*. Despite the profound defect of the innate immune response, MyD88 deficiency allows the emergence of an adaptive immunity. These data demonstrate that activation of multiple TLRs contributes to an efficient innate response to mycobacteria, while MyD88-dependent signalling is dispensable to generate adaptive immunity.

Background: Tuberculosis, immune response and Toll-Like receptors

Infectious tuberculosis due to *Mycobacterium tuberculosis* (MTb), an intracellular pathogen capable of surviving and persisting within host mononuclear cells, represents a major global health challenge. A coordinated response of cells of the innate and adaptive immune system is required to control infection (Flynn and Chan, 2001; North and Jung, 2004). The pathogen sequestered within macrophages within granuloma, a dynamic structure containing activated lymphocytes and macrophages contains the spread of infection. Gene targeted mice and neutralising antibodies demonstrated critical mediators controlling *M. tuberculosis* infection, including IFNγ, IL-12, IL-23, TNF, lymphotoxins, CD40 and nitric oxide (Flynn and Chan, 2001; North and Jung, 2004). Mycobacteria have evolved to resist the eradication by macrophages by using elaborate evasion mechanisms (Flynn and Chan, 2003). In a healthy host a subclinical latent or chronic infection may persist (Gomez and McKinney, 2004). Neutralisation of IFNγ or TNF, inhibition of iNOS, or T cell depletion leads to reactivation of latent infection (Botha l, 2003).

Continous macrophage and T lymphocyte activation control the viable, but sequestered bacilli within phagocytes in the granulomas structure. We hypothesise that mycobacterial products released by the sequestered bacilli, such as glycolipids PIMs, LM, LAM, lipoproteins and other mycobacterial factors,

may contribute to continued macrophage and dendritic cell activation through pathogen pattern recognition receptors (PRR) such as Toll like receptors (TLR) and others.

Mammalian TLRs represent a structurally conserved family of membrane receptors, which have homology to the Drosophila Toll system (Akira and Takeda, 2004; Akira et al., 2006; Medzhitov et al., 1997). Microbial products activate mammalian TLRs inducing gene transcription regulating the adaptive immune response, resulting in chemokines, cytokines and costimulatory molecules. The TLR family comprises now eleven members (Quesniaux and Ryffel, 2004).

The mycobacteria cell wall is composed different glyocolipids such as LAM, mycolic acid, lipopeptides, phosphoinositol, which may be recognised by the immune system (Chatterjee and Khoo, 1998; Daffe and Draper, 1998). So far, TLR2, TLR4 and, TLR1/TLR6 that heterodimerize with TLR2, have been implicated in the recognition of mycobacterial antigens. LMs, the biosynthetic precursors of LAMs, represent another class of abundant pro-inflammatory molecules of the mycobacterial cell wall. We showed that LMs from various mycobacterial origins are potent activators of proinflammatory cytokines in macrophages requiring TLR2 signalling (Quesniaux et al., 2004b). Based on the present knowledge the balance between PIM, LM and LAM synthesis by pathogenic mycobacteria might provide pro- or anti-inflammatory, immunomodulatory signals during primary infection but also during latent infection.

Mycobacterial lipoproteins were shown to activate APC through TLR2 signalling. However, the 19kD lipoportein, LpqH (Rv3763) has also TLR2 dependent, inhibitory functions on IFNγ regulated responses, including MHC-II antigen processing in macrophages. For more details on mycobacterial ligands, their receptor specificity and biological properties see (Quesniaux et al., 2004a; Quesniaux et al., 2004b).

TLR dependent activation of macrophages and dendritic cells

Using bone marrow derived macrophages derived from TLR2 and/or TLR4 deficient mice, we showed TLR2- and to a lesser extent TLR4-dependent activation of TNF and IL12 production after infection with live *M. bovis* BCG (Fremond et al., 2003; Nicolle et al., 2004b), which is completely abrogated in MyD88 deficient macrophages (Nicolle et al., 2004a). However, recognition of heat-killed *M. bovis* BCG, extensive-freezed-dried *M. bovis* BCG, a soluble fraction of *M. bovis* BCG culture supernatant, or a “well dispersed” live *M. bovis* BCG cultivated in the presence of detergent, was predominantly mediated through TLR2, as essentially no response remained in TLR2-deficient macrophages or dendritic cells (Nicolle et al., 2004b). Interestingly, the expression of CD40, CD80 and CD86 on macrophages and dendritic cells was not affected by the absence of single TLRs or the TLR signalling adaptor protein MyD88 suggesting a normal costimulation of T cells (Fremond et al., 2004; Nicolle et al., 2004a). In summary, these results suggest that purified mycobacterial antigens and whole bacilli preferentially interact with TLR2 and TLR4, possibly in combination with additional TLRs and PRRs, leading to MyD88 dependent activation of antibacterial effector pathways.

Role of TLR/MyD88 signalling in the host response to TB infection

Using single TLR deficient mice we investigated their susceptibility to low dose aerosol infection with

virulent *M. tuberculosis* H37Rv. TLR4 deficient mice displayed reduced bacterial clearance during a long-term infection protocol and develop a chronic pneumonia and died within 15 weeks (Abel et al., 2002). The data were confirmed recently by an independent group (Branger et al., 2004). In short term infections, no significant differences in the inflammatory response or the bacterial burden in infected organs during the first 50 days of infection and long-term were found (Kamath et al., 2003; Reiling et al., 2002; Shim et al., 2003). CD14, a coreceptor of TLR4, appears not to be involved in host resistance, as CD14 deficient mice clear normally the infection. Then we investigated the role of TLR2 in the host response, and infected TLR2 deficient mice by aerosol using 500 CFU. TLR2 deficient mice initially control an aerosol infection with signs of T cell activation, but develop increased bacterial burden and chronic pneumonia with death in 5 months (Drennan et al., 2004). Therefore, the data suggest that TLR2 may function as a regulator of inflammation, and in its absence an exaggerated immune-inflammatory response develops.

Finally, we investigated the role of the common TLR adaptor molecule myeloid differentiation factor 88 (MyD88) and infected MyD88 deficient mice with *Mycobacterium tuberculosis* (Fremond et al., 2004). Aerogenic infection of MyD88 deficient mice with MTB was lethal within 4 weeks as shown for TNF deficient mice. Mice succumbed to acute necrotic pneumonia with 2 log higher CFU in the lung. This was associated with acute, necrotic pneumonia, despite a normal T cell response with IFNγ production to mycobacterial antigens upon ex vivo restimulation. Fatal infection in MyD88 deficient mice is not surprising in view of the profound defect of innate immunity of MyD88 deficient mice (Shi et al., 2003).

We then asked whether MyD88 deficient mice are able to develop an adaptive immune response. In view of the fact that MyD88 deficient macrophages upregulated costimulatory molecules such as CD40 and CD86 (Nicolle et al., 2004a), we investigated the response to BCG vaccination. We found comparable antigen specific responses with IFNγ production in the absence of MyD88 as in wild-type mice. Furthermore, prior BCG vaccination conferred a substantial protection in vaccinated MyD88-/- mice from acute MTB infection with 2 log reduced mycobacterial load (CFU) in the lung as compared to non-vaccinated mice (Fremond et al., 2004). These data demonstrate that MyD88 signalling is dispensable to raise an acquired immune response, which however is not sufficient to compensate the profound innate immune defect of MyD88 deficient mice to control MTB infection. The present in vivo evidence suggests that signalling through single TLRs has only a modest effect in acute mycobacterial infection, while abrogation of most of TLR signalling as found in MyD88 deficient mice results in profound deficiency of innate immunity with preserved adaptive immunity.

Conclusion

The present results support the emerging paradigm that at least TLR2 and TLR4 play a role in sensing mycobacteria and mounting an antimycobacterial immune response in vitro and in vivo. In future, the repertoire of identified mycobacterial antigens, which mediate TLR signalling, are likely continue to increase. The combinatorial recognition of pathogen-associated molecular patterns by more than one TLR, and crosstalk between TLRs and other PPRs signalling opens a new dimension (Underhill, 2003).

A more detailed knowledge of the stimulatory and inhibitory ligands of TLR and PRR might allow (Janeway and Medzhitov, 2002) modulating the immune response to mycobacteria. In summary, the common TLR adaptor molecule MyD88 is critical to develop a robust host response to MTb infection, and the profound defect of innate immunity is not compensated by other PRR molecules. Interestingly, MyD88 deficiency allows the emergence of an adaptive immunity. Therefore multiple TLRs contribute to an efficient innate response to mycobacteria, while MyD88-dependent signalling is dispensable for adaptive immunity.

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