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Review

TLR4 in Toxoplasmosis; friends or foe?

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ABSTRACT

Toxoplasma species are obligate intracellular protozoan which are responsible for induction of several forms of Toxoplasmosis in humans. The mechanisms responsible for the progression of the prolonged forms of Toxoplasmosis and associated pathologies are yet to be identified. However, previous studies proposed that immunological and genetic parameters may play important roles in the etiology and complexity of Toxoplasmosis. Pathogen recognition receptors (PRRs) recognize microbial antigens and induce immune responses against parasites, including toxoplasma species. Toll like receptors (TLRs) are PRRs which recognize toxoplasma as a pathogenic parasite and activate immune cells. It has been reported that the TLR4 is a critical innate immune cell receptor in toxoplasma detection and subsequently activates immune responses using either MYD88 or TRIF pathways. This review collates recent information regarding the role of TLR4 and its related signaling molecules with Toxoplasmosis.

1. Introduction

The zoonotic disease of Toxoplasmosis is caused by the intracellular protozoan *Toxoplasma gondii* (*T. gondii*) [1]. Oral ingestion of parasite oocysts and tissue cysts is the route of transmission to human intermediate hosts [2]. The infection in immunocompetent persons is typically chronic and asymptomatic, in which the parasites transform from a motile form to quickly multiplying tachyzoites and then on to slow growing bradyzoites in tissue cysts [3,4]. In both acquired and genetically immunodeficient humans, the parasite can invade nucleated cells of many tissues and causes severe conditions such as chorioretinitis and encephalitis in adult hosts, as well as hydrocephalus and abortion of fetuses [2]. Immune responses against *T. gondii* in immunocompetent individuals can clear the acute form of parasites (i.e. tachyzoites) from infected tissues or shift the parasite to inactive bradyzoites in tissue cysts [5]. The differences in mechanisms responsible for the development of acute Toxoplasmosis in some humans and latent *Toxoplasma* infection or its eradication in others are not yet clear. However, there is some evidence that suggests immunological and genetic conditions may be involved in the pathogenesis of Toxoplasmosis and its complications.

Toll-like Receptors (TLR) are intra/extra-cellular immune cell receptors that belong to the Pathogen Recognition Receptors family (PRR) and play crucial roles in microbial and parasite Pathogen Associated Molecular Patterns (PAMP) and Damage Associated Molecular Patterns (DAMP) recognition and the subsequent immune responses induced against infectious agents such as *T. gondii*. The first TLRs were described in 1997 by Janeway and Medzhitov [6] and constitute a large family of proteins with human immune cells expressing TLR1-10 [7]. TLRs are similar in intra and extracellular domains. However, they recognize a spectrum of microbial macromolecule PAMPs such as proteins, lipids, DNA, and RNA [8]. Microbial PAMPs including lipopolysaccharide, lipopeptides, bacterial flagellin, viral dsRNA, viral or bacterial ssRNA and CpG-rich unmethylated DNA are recognized by TLR4, TLR2 in combination with TLR1 or TLR6, TLR5, TLR3, TLRs 7/8 and TLR9, respectively [8].

Abbreviation: AP-1, Activator protein 1; DAMP, Damage associated molecular patterns; DNMT-1, DNA methyltransferase 1; IRAK1, Interleukin-1 receptor associated kinase-1; MAPK, Mitogen-activated protein kinase; MHC, Major histocompatibility complex; miRNAs, MicroRNAs; MYD88, Myeloid differentiation primary response; NADPH, Nicotinamide adenine dinucleotide phosphate; NF-κB, Nuclear factor kappa-light-chain-enhancer of activated B cells; PAMP, Pathogen associated molecular patterns; PBMC, Peripheral blood mononuclear cell; PRR, Pathogen recognition receptors; RIP1, Receptor-interacting protein 1; TAK1, Transforming growth factor b-activated kinase 1; TIRAP, TIR domain-containing adapter protein; TLR, Toll like receptor; TRAF6, TNF receptor associated factor; TRAM, TRIF-related adapter molecule; TRIF, TIR-domain-containing adapter-inducing interferon-β.

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These combinations of TLR receptor complexes are ligand specific and stimulate immune responses against opportunistic protozoan such as *T. gondii*. TLR/PAMP or DAMP interactions initiate a variety of immune cells functions including migration [9], phagocytosis [10], inflammatory cytokine secretion [11] and NADPH oxidase activation [12]. As a result, TLR4 activation may result in the expression of MHC, inflammatory cytokines and homing molecules, through the recognition of extra/intra-cellular *T. gondii* DAMPs in both TRIF and MYD88 dependent manners [13]. Therefore, a deficiency in TLR4 expression may suppress immune responses against *T. gondii*. The aim of this review article was to collate the recent findings on the potential relationship between TLR4 and *Toxoplasma* pathogenesis.

2. Toxoplasmosis

T. gondii (Apicomplexa; Sarcocystidae) was discovered in 1908 at the Pasteur Institute in Tunis by Charles Nicolle and Louis Manceaux. This obligate coccidian parasite utilizes felids and other warm-blooded animals as definitive and intermediate hosts, respectively [14,15]. In humans, *T. gondii* causes a common disease known as Toxoplasmosis [14]. This infection in humans has a world prevalence ranging from 25 to 30 percent [16]. The prevalence of Toxoplasmosis varies in different geographical areas, ranging from 10 to 30 percent in north America, northern Europe, south east Asia and Sahelian countries of Africa, with a moderate prevalence of 30–50 percent reported in central and southern Europe and a high prevalence of the infection reported in Latin America and tropical African countries [17]. Many other animals including livestock, pigs, canids, felids, wild cervids, marsupials, marine mammals, monkeys and ungulates are commonly infected with *T. gondii* and may show clinical and subclinical manifestations of the infection [16]. There are some environmental parameters that may affect the infection status in human populations, such as the survival of oocysts in the soil resulting from climatic factors, for example, tropical countries with a humid and warm climates generally have a higher prevalence of Toxoplasmosis [18]. Infection rates in meat-producing animals also impinges on human infection [19]. Moreover, anthropogenic factors such as economic, social, dietary and cultural variables can influence on the Toxoplasmosis prevalence [20]. Since the efficiency of immune system is significantly correlated with genetics, age and environmental parameters it is not surprising that Toxoplasmosis is more prevalent in older age groups in some countries [20]. Although environmental parameters are a risk factor for infection, defects in the recognition of pathogens and activation of the immune system also influence infection and clearance from the human host. PRRs are important in parasite recognition, and the pathogenesis of Toxoplasmosis. The next section describes TLR4 as an important PRR.

2.1. Introducing of TLR4

TLR4, also known as ARMD10 or CD284, is a receptor for some microbial PAMPs, which are discussed below. The TLR4 gene is located on 9q33.1 and is strictly conserved, as are the other TLRs [21]. The TLR4 molecule consists of three domains including extracellular leucine-rich repeats (which participate in PAMPs recognition), hydrophobic transmembrane and cytoplasmic Toll/interleukin-1 receptor (TIR) domains (Fig. 1). TLR4 recognizes PAMPs in a homodimer format which can activate MYD88 and TRIF intracellular signaling pathways (See next sections).

2.2. TLR4 ligands and signaling

Interactions of TLR4 with various ligands can induce the activation of both TRIF and MYD88-dependent intracellular signaling pathways [22]. The ability of TLR4 to utilize TRIF and MYD88 adapters is unique among the TLRs. This property may cause the induction of pro-inflammatory cytokines including IL-6, TNF-α and type I interferons [22]. In the MYD88 dependent pathway, TLR4-MD-2 dimerization at the cell surface leads to recruitment of two adapter proteins, namely TIRAP (MAL) and MYD88, which can activate NF-κB, AP-1 and IRF5 as pro-inflammatory transcription factors and consequently pro-inflammatory cytokines are produced [23]. Furthermore, TLR4 molecules can enter endosomes and utilizes TRAM and TRIF to activate another transcription factor, IRF3, that in turn, can induce the production of type I interferons (Fig. 1) [24]. Lipopolysaccharide (LPS) is the most important ligand of TLR4/CD14/MD2 complex. LPS binding protein (LBP) binds secreted LPS molecules to CD14 (a glycosylphosphatidylinositol-anchored protein) and then CD14 transfers the LPS to MD2. MD2 is a soluble protein bound to the TLR4 extracellular domain by a non-covalent bond. The LPS/MD-2 complex causes a conformational change in MD-2 which leads to binding of the MD-2-TLR4 complex to a second TLR4 receptor. TLR4 homo-dimerization triggers intracellular signaling (See next section). In addition to LPS, some other ligands are also recognized by TLR4 including high-mobility group box-1, hyaluronan, heat shock protein 60, free fatty acids as endogenous ligands, allergenic nickel and the adjuvant monophosphoryl lipid A (MPLA) [25,26]. In addition, GLA-SE is a novel emulsified synthetic TLR4 ligand [27]. These TLR4 ligands are able to either activate TLR4 directly or bind to and transport LPS, and as a result, the sensitivity of cells to LPS is enhanced. Therefore, many of the endogenous TLR4 ligands may play significant roles in PAMP binding/sensitizing molecules [28]. TLR4 interactions with its ligands recruits TIR-containing adapters such as TIRAP and activation of the MYD88-dependent pathway whereas TRIF and TRAM molecules are essential in the induction of TLR4-TRIF-dependent signaling pathway [29]. In the MYD88-dependent signaling pathway, the interaction between TLR4 and its ligand at the cell plasma membrane induces binding of TIRAP and the subsequent recruitment of MyD88, as an adapter protein. In turn, recruited MYD88 is capable of activating several intracellular signaling molecules including IRAK4, IRAK1, TRAF6, TAK1 and subsequently pro-inflammatory transcription factors such as NF-κB, AP-1 and IRF5 which induce the transcription from genes of several pro-inflammatory cytokines in cell nucleus (Fig. 1) [22]. Interestingly, translocation of TLR4 to the endosome and interaction with its ligands in the surface of endosome membrane can trigger the TRIF-dependent signaling pathway, in which TRAM binds to the TIR domain of TLR4 [30]. The adapter protein, TRIF, is utilized to bind TRAM and to activate IRP1 and TRAF3 molecules via their phosphorylation [30]. Phosphorylated TRAF3 can activates IRF3 and translocate it into the nucleus to induce the transcription of type I interferons, while, the phosphorylation of RIP1 induces the activation of other transcription factors such as NF-κB and AP-1 (Fig. 1) [31].

2.3. TLR4 and toxoplasmosis

Based on the important roles played by TLR4 in the recognition of microbes and activation of appropriate immune responses, it appears that TLR4 may be important in the pathogenesis of Toxoplasmosis and potentially in the impaired immune responses observed in some infected patients. Furuta et al., demonstrated that TLR4 is important for the induction of small intestine innate immune responses against *T. gondii* infection [32]. It has also been demonstrated that *T. gondii*-derived glycosylphosphatidylinositols leads to activation of immune cells in MYD88 dependent manner [33]. Niehus et al., showed that glycosylphosphatidylinositols of *T. gondii* can induce human macrophages to synthesize matrix metalloproteinase-9 (MMP9) via the binding and subsequent