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TOLL LIKE RECEPTORS: THE IMMUNOMODULATORY AGENTS AND NOVEL TARGET OF IMMUNOTHERAPEUTIC RESEARCH

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ABSTRACT

Toll-like Receptors (TLRs), the evolutionarily conserved molecules are identified as the pattern recognition receptors (PRRs) in vertebrates and invertebrates which recognize the pathogen-associated molecular patterns (PAMPs). The TLRs signaling is via interactions with adaptor proteins including MyD88 and toll receptor associated activator of interferon (TRIF). They directly detect the pathogen invasion and induce either immuno-stimulatory or immunomodulatory biological response. This ability of TLRs to modulate the immune system has been taken in concern in recent studies to develop an adaptive immunotherapy against cancer and several other neurological disorders. Moreover, to trace their auxiliary therapeutic effects, the TLR agonists are now undergoing the extensive clinical investigation. This review discusses the therapeutic potential of TLRs as Immunostimulators and Immunomodulators. Alongside, the association of TLRs with autoimmune responses and human diseases is also explored.

Keywords: TLR, PRS, PAMP, Targeted therapy, autoimmune response

INTRODUCTION

The Toll-like receptors are so named because of their similarity to the *Drosophila* Toll receptor (Leimaitare *et al.*, 1996). They recognize the pathogen-associated unique molecules such as the bacterial cell wall components peptidoglycan (TLR2) and lipopolysaccharide (TLR4) (Zhang *et al.*, 2004). The dsRNA, ssRNA and nonmethylated Cytosine-Guanosine (CpG) DNA are also identified by TLR3, TLR7 and TLR9 respectively (Akira and Takeda,

2004; Takeda *et al.*, 2003) (Fig.1). As a consequence, after activation of TLRs by said ligands the different cytokine and chemokines are produced which can further initiate the local inflammatory response to provide a first-line defense against pathogen invasion. The TLRs are mostly located on antigen presenting cells. Each of them, with the exception of TLR3, signals through the MyD88 (myeloid differentiation88 gene) dependent pathway, initiated by the MyD88 adaptor protein (Broad *et al.*, 2007). The MyD88 recruitment at TLRs instigates the formation of Interleukin-1 (IL-1) receptor associated kinase (IRAK) complex (Fig.2). Four members of this family have been

identified: IRAK-1, IRAK-2, IRAK-4 and IRAK-M (Björkbacka, 2006). Knockout mice for IRAK-4 had completely abolished responses to TLR2, TLR3, TLR4 and TLR9 showing that this molecule is essential for TLR signaling (Pasterkamp *et al.*, 2004). Also, the MyD88 deficient cells show the increased expression of costimulatory molecules, such as CD80 and CD86 to induce dendritic cell maturation in the absence of MyD88 dependent pathway. Formation of IRAK complex further result in phosphorylation of IKK α /b, activation of the transcription factors NF κ B, IRF1, and IRF7, and generation of the pro-inflammatory cytokines IL-6 and TNF α and others (Takeuchi *et al.*, 200; Brenda *et al.*, 2008). While TLR3 signals through the MyD88 independent pathway, initiated by the TRIF adaptor molecule (Broad *et al.*, 2007) which unlike to others initiates the phosphorylation of IKK ϵ and activates the transcription factors IRF3 and IRF7 to generate the anti-viral molecules such as Interferon β (IFN β) (Brenda *et al.*, 2008). Amongst TLRs, only TLR4 can utilize either of these pathways (Fig-2).

The TLRs are proved to be a key link between obesity, insulin resistance, and cardiovascular disease. Pharmacologic approaches to modify the activity of the TLRs may therefore have favorable influences on the atherosclerotic disease process and other cardiovascular disorders (Boekholdt *et al.*, 2008).

TLRs AND T_H RESPONSE

Since, TLRs are widely expressed on immune cells such as Dendritic Cells (DCs), monocytes, mast cells, neutrophils, B cells, endothelial cells and fibroblasts therefore; they activate these cells to produce IL-12 and IFN- α to mount the Th1 responses. However, apart from the professional antigen presenting cells

(APCs), TLRs are also found expressed on non-immune cells such as mesangial, astrocytes, uterine epithelial and fibroblasts cells (Lutz and Schuler 2002).

Role of TLRs in the development of classic Th2 responses is still debatable. Though, highly purified *P. gingivalis* LPS, a putative TLR2 ligand (Methe *et al.*, 2005) Pam-3-cys, a synthetic TLR2 ligand and Schistosomal Egg Antigen (SEA) have been shown to induce the Th2 responses (Sakata *et al.*, 2007).

TLRs AS IMMUNOSTIMULATORS

TLRs being organism-wide sensor system play a frontline effector role in host defense. Hitherto, many TLR agonists have been developed to be used as vaccine adjuvants to stimulate & enhance the antigen specific memory response and circumvent the booster burden. Recently, the utilization of TLR agonists against neural cancer has been an emerging strategy to stimulate the tumor specific natural adaptive anti-tumor immune response. In this approach, specific tumorous area is only targeted along with protection of normal brain structures. Several TLR agonists have been investigated in this regard.

One particular candidate the *Imiquimod* has got most attention in cancer immunotherapy. It is a synthetic TLR7 agonist that has been given the Food & Drug Administration (FDA) approval as a topical treatment for Herpes Sarcoma Virus (HSV)-2 lesions (Prins *et al.*, 2006). In a recent study, the topical application of *Imiquimod* has been shown to decrease intracranial tumor burden in a malignant melanomic mouse model (Prins *et al.*, 2006). It is believed that the said effect was shown due to prolonged existence of antigen-pulsed dendritic cells and improved CD8⁺ priming. However, a prolonged

Imiquimod treatment has also been reported the increased hemorrhaging and inflammation-induced mortality.

Lipopolysaccharide (LPS), a potent agonist of TLR4 has also been investigated for its anti-tumor immune efficacy. In a study by Won *et al* (2003), the LPS have been shown to decrease the mean tumor mass of primary subcutaneous Glioblastoma Multiforme (GM) tumors in induced mouse model. Supportively, the LPS administered mice bearing intracranial GM tumors have been shown the increased survival than TLR4 knockout mice of the same group. The observations suggested that the LPS cytotoxicity is however insufficient to clear the tumors but certainly may act as a tumor-specific immune adjuvant (Chicoine *et al.*, 2007).

The chemotherapeutic use of one more agonist of TLR9, the nonmethylated CpG oligodeoxynucleotides have also got attraction and shown the great promise. It has been demonstrated that the intratumoral CpG administration in mice and rats with subcutaneous and intracranial neuroblastoma tumors increases the survival time and protects against secondary tumor challenge (Carpentier *et al.*, 1999). It induces the apoptosis of intracranial glioblastoma cells (El Andaloussi *et al.*, 2006). TLR9 is chiefly expressed by glial cells in mice but restricted to the B cells and plasmacytoid dendritic cells in healthy humans subjects (Iwasaki *et al.*, 2004). A Phase II clinical trial is in progress to explore the therapeutic potential of said ligand into generation of a specific anti-tumor immune response in patients with brain tumors.

TLRs AS IMMUNOMODULATORS

In addition to immunostimulation, immunosuppressive properties of TLRs are also exploited to develop immunotherapy

against neurological disorders. Such immunosuppressive therapies are based on the phenomena of “tolerance”, where moderate pre-exposure of deleterious stimulus potentiate the animals to show the tolerance against subsequent intensive exposure of the same. For instance, the mild pretreatment of LPS can protect an animal from detrimental effects during subsequent higher exposure of the same. Supportively, a study by Tasaki *et al* (1997) demonstrated that the systemic administration of low dose LPS subsequently render the tolerance in hypertensive rats to ischemic brain damage induced by middle cerebral artery occlusion (MCAO). The mechanism behind is believed that the inflammatory response induced during pretreatment of LPS enhances the blood perfusion towards ischemic area at the time of ischemia and afterwards (Kunz *et al.*, 2007; Dawson *et al.*, 1997).

Moreover, LPS pretreatment has also been reported to protect brain against cytotoxic effects of TNF α after cerebral ischemia (Rosenwieg *et al.*, 2004). It is observed that the LPS tolerant Cells possess deficient ability to generate TNF α in response to TLR4 activation. These cells unlike to naïve cells, do not recruit MyD88 to TLR4, and fails to activate IRAK-1 and NF κ B thereby leading to blockage in TNF α expression (Medvedev *et al.*, 2002). Since, Dendritic Cells (DC) contain TLR9 thus it is observed that after exposure of DCs from TLR9 agonists (with or without antigen), various pro-inflammatory cytokines and chemokines were secreted which further activated and some time suppressed (caused due to specific cytokine viz. IL-6) to the effector T cells (Th1 and/or CTL cells) response (Pasare and Medzhitov, 2003) (Fig 3).

ROLE OF TLRs' IN AUTOIMMUNE RESPONSE

The autoimmunity is a consequence of unbridled proliferation of auto-reactive immune cells along with defects in the maintenance of tolerance (Yamada *et al.*, 2005). Several evidences have now established that TLRs contribute to the development of autoimmunity (Suzuki *et al.*, 2002). The virus induced IFN- α , whose effect can be mediated via TLRs 3, 7 and 9, contribute significantly in the pathogenesis of type I diabetes mellitus and Systemic Lupus Erythematosus (Kobayashi *et al.*, 2002). Moreover, viral dsRNA (double stranded RNA) can bind to TLR3 and activate NF- κ B which induces the formation of apoptotic bodies that further promotes plasmacytoid DCs to produce IFN- α (Kawai, 2001). B cells as professional antigen presenting cells express both TLR7 and TLR9. Out of which, TLR 9 are reported to break the tolerance of auto-reactive B cells through binding to hypomethylated CpG-containing DNAs, which are present in the mammalian DNA itself (Doyle *et al.*, 2002). These regions, which may be the derivatives of apoptotic or necrotic cells, form a complex with anti-DNA IgG autoantibodies and stimulate B cells via BCR and TLR 9 (Kaisho *et al.*, 2001).

POSSIBLE ROLE OF TLRs IN HUMAN DISEASES

TLRs being active immunomediators recognize the various endogenous proteins that are often released in response to stress or tissue damage and variably found at the sites of chronic inflammation. These may be heat shock proteins (hsp) 60, 70, 96, extra domain A-containing fibronectin fragments, hyaluronan fragments, fibrinogen, β -defensins, oxidized low-density lipoprotein and heparan sulfate

(Leadbetter *et al.*, 2002). This ability of TLRs to interact with inflammatory cytokines has caused them to be investigated for their role in Rheumatoid Arthritis, Crohn's disease, Ulcerative Colitis, Psoriasis, Chronic Obstructive Pulmonary Disease (COPD) and Atherosclerosis (Anders *et al.*, 2005). As evidence, the polymorphisms in TLR9 have been shown to be associated with an increased risk of Systemic Lupus Erythematosus (Seibl *et al.*, 2004). In a study by Lynn *et al.* (2003) the TLRs inhibition with hsp10 has suggested the beneficial effect on the symptoms of rheumatoid arthritis (RA). Moreover, mutated TLR4 have been found associated with a decreased response to inhaled lipopolysaccharide and increased susceptibility to severe bacterial infections and bacteremia (Kawakami *et al.*, 2008).

Since, the numbers of TLRs are involved in different types of infection and each has differing immune and inflammatory cascades therefore, mutations in these genes have provided the subject to study the body's response to endogenous and exogenous ligands (Steinman, 2006). In future, these may be the molecular targets which can ably interfere to the development of septic shock syndrome, while maintaining some natural immune response to pathologic bacteria (Doherty *et al.*, 2006).

CONCLUSION & FUTURE PERSPECTIVES

Above discussion has demonstrated the TLRs as an immunomodulatory agent who can generate the protection against cancer and suppress the damaging inflammatory responses. Captivatingly, the ability of TLRs to activate tumor specific lymphocytes has been translated into antitumor immunotherapy in recent studies.

Also, the TLR agonists based immunostimulation has been proven its success in treating the neurological disorders. Though, owing to systemic cytokine induction, the systemic use of TLR agonists may be restricted by dose-limiting toxicities but combinatorial approaches with conventional therapies such as chemotherapy may be promising. However, pharmacological interventions using TLRs antagonists are in infancy but hold much promise for future success. Therefore, with increasing evidences of active role in initiation and maintenance of immune response, the TLRs can be an attractive drug targets for regulating the immune terror.

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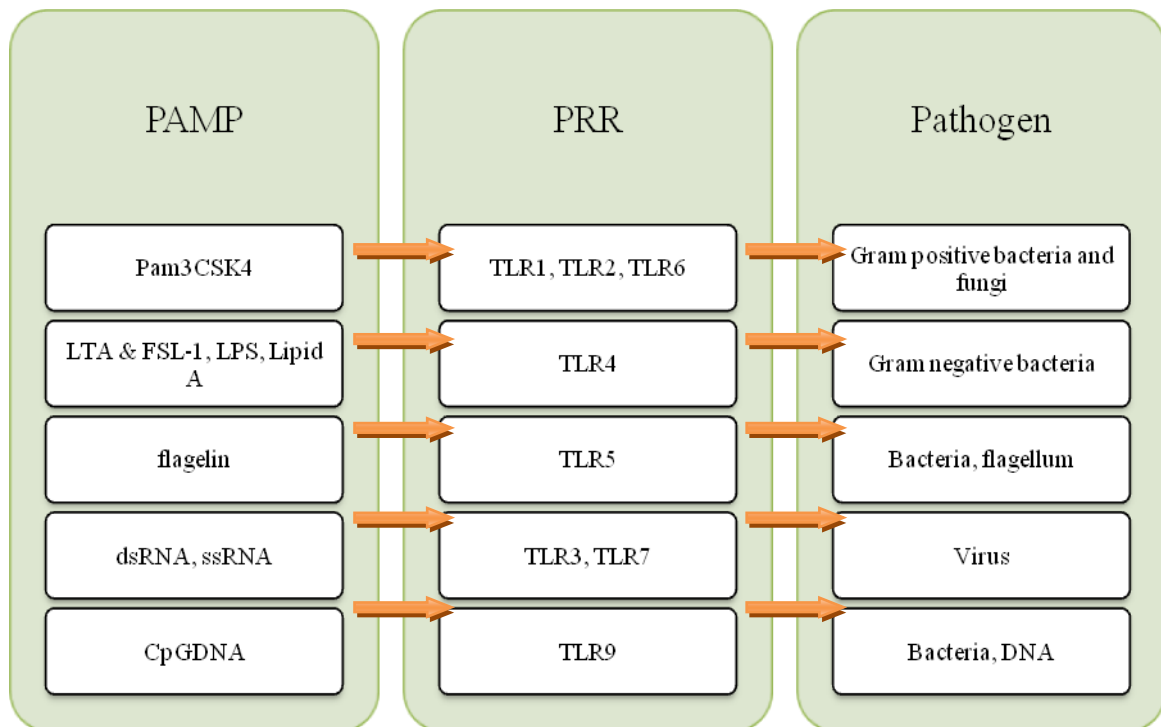


Fig. 1: Relational representation of Pathogen-Associated Molecular Pattern (PAMPs) with Pattern Recognition Receptors (PRRs) and pathogens.

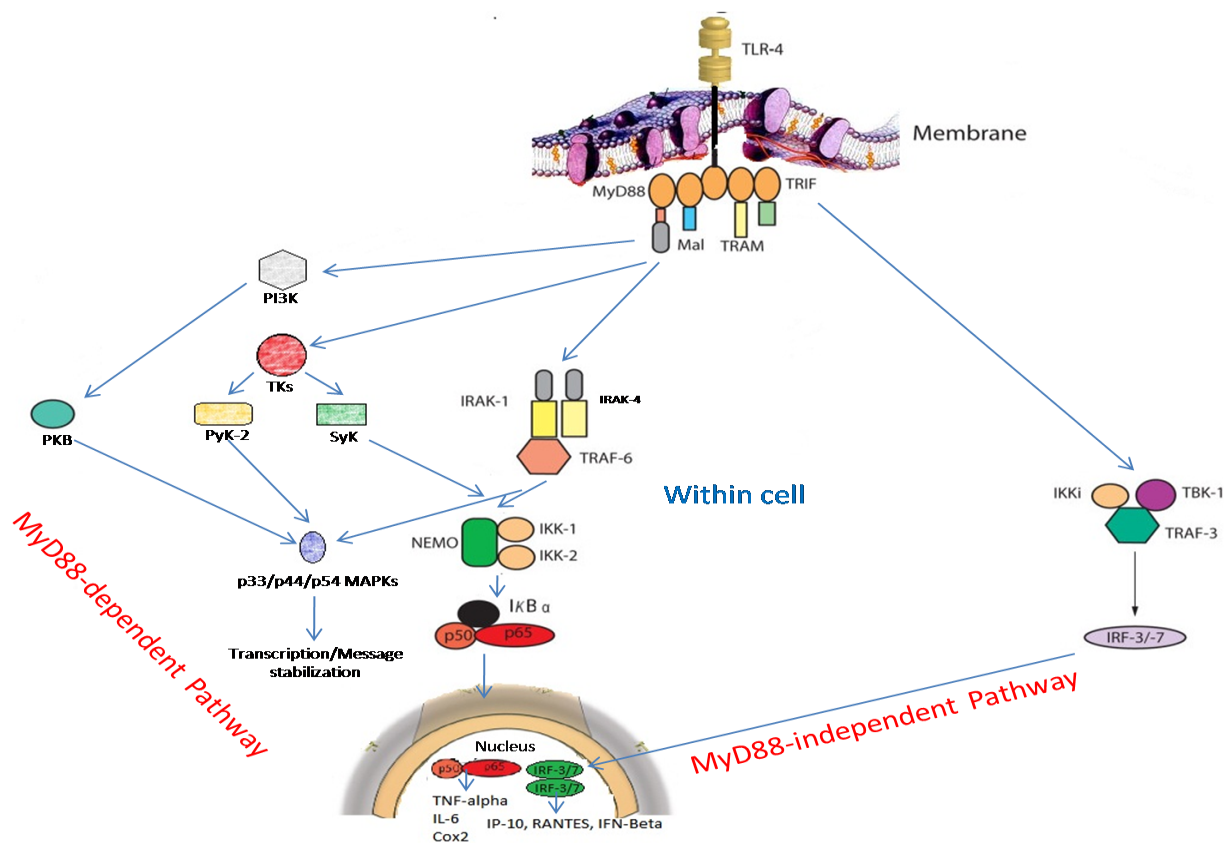


Fig. 2: MyD88 dependent and independent pathway of TLR signaling.

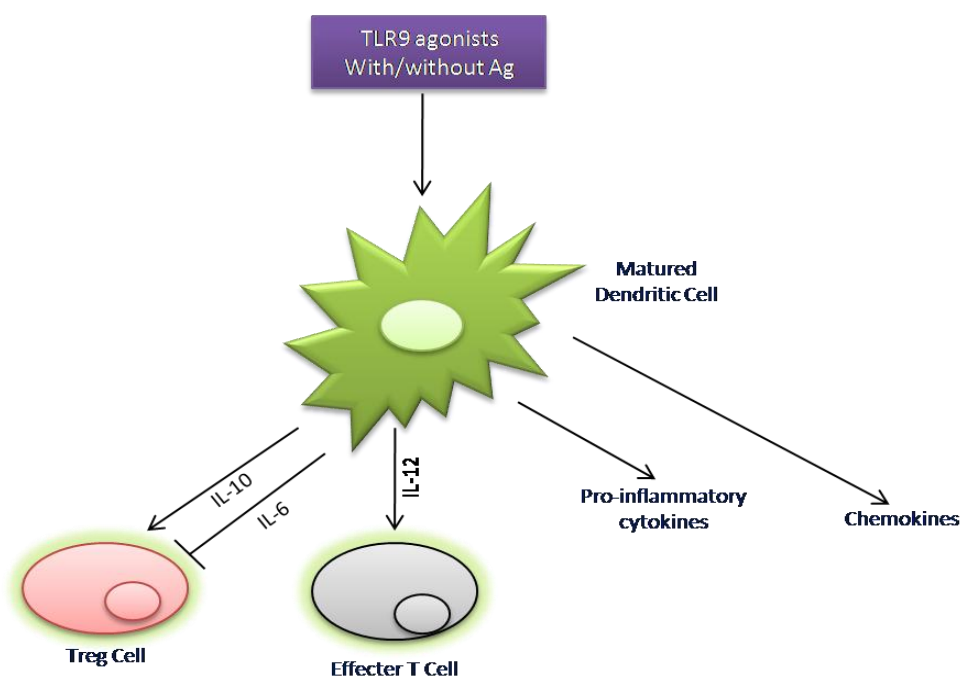


Fig. 3: TLR9 mediated cytokine and chemokines secretion from Dendritic Cells and consequent effector and suppressive immune response.