Immunotherapy Insights

CONSISE REVIEW

"Toll" Extending Its Gate from *Drosophila* Development to T Cell Response: Implication in Innate Immunity, Adaptive Immunity and Immunotherapy

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Abstract: "Toll" protein was originally discovered as a developmental marker in fruit fly (*Drosophila*). Now Toll like receptor (TLR) is envisioned as one of the important innate immune group of receptors regulating mammalian immune system. Interestingly, TLR response has been translated in immuno-pathology of most of the diseases and its immune responses including tumor immunity, infection immunity and autoimmunity. Moreover, in very recent time, TLR response has been suggested to modulate cell mediated immunity (CMI). Accordingly, the new paradigm of TLR response in T cell proposes a challenging work of T cell biology, both in basic and in translational research. Here we have reviewed the structural and functional homology of "Toll" protein in *Drosophila* and mammalian TLR, role of TLR in innate immunity, adaptive immunity and immunotherapy, recent updates of TLR response in T cells and the yet unanswered questions on the role of TLR in T cells to explore the new paradigm of TLR as one of the important connecting bridges between innate and adaptive immunity.

Keywords: TLR, APC, T cell, immunity

Immunotherapy Insights 2011:2 1–14

doi: 10.4137/INT.S7422

This article is available from http://www.la-press.com.

Introduction

TLRs acquired their name from their similarity to the protein coded by the Toll gene, first identified in *Drosophila* in 1985 by Christiane Nüsslein-Volhard.[1] It was an important breakthrough that *Drosophila* protein Toll had a cytosolic domain homologous to IL-1RI sequence (homologous partner of TLR).[2] Although extracellularly different, all of them have leucine-rich repeats and their signaling domains are similar with whole stretches of conserved amino acids. This was really exciting for the scientific world how a protein having such a sequence and structural similarity with major host defence molecules, could be involved in determining dorsoventral polarity, a process apparently far different from inflammation. However, Toll was known to regulate a transcription factor termed 'Dorsal' which is a member of the NF-κB family on the basis of homology and function. Toll activates 'Dorsal' via a protein kinase called 'Pelle'.[3] Pelle is homologous to the IL-1 receptor-associated kinase,[4] a key signaling molecule for TLRs or IL-1 receptor family proteins. In 1994, Witham and his colleagues reported a similar signaling domain like IL-1RI and Toll that is popular as TIR (Toll/IL-1R resistance domain) domain and is required for resistance to tobacco mosaic virus in tobacco as TIR was reported homologous to Toll.[5] In 1996, Toll was documented to be important for disease resistance in *Drosophila*, particularly for resistance to fungal pathogens.[6] In 1997, human Toll was first reported and it was shown by transfection of active mutant of human Toll in human cell lines. It can induce activation of NF-κB which induces proinflammatory cytokines and expression of B7.1.[7] In 1998, a family of human receptors structurally related to *Drosophila* Toll were reported within the IL-1R/TLR super family of human Toll-like receptors (hTLRs).[8] Although at that time there were no ligands known for TLRs, the discovery of structure, function and its conservation from unicellular to plants and many other species was an important achievement in the field of TLR biology. A comparative homology of *Drosophila* 'Toll' and mammalian "TLR" has been depicted in Figure 1.

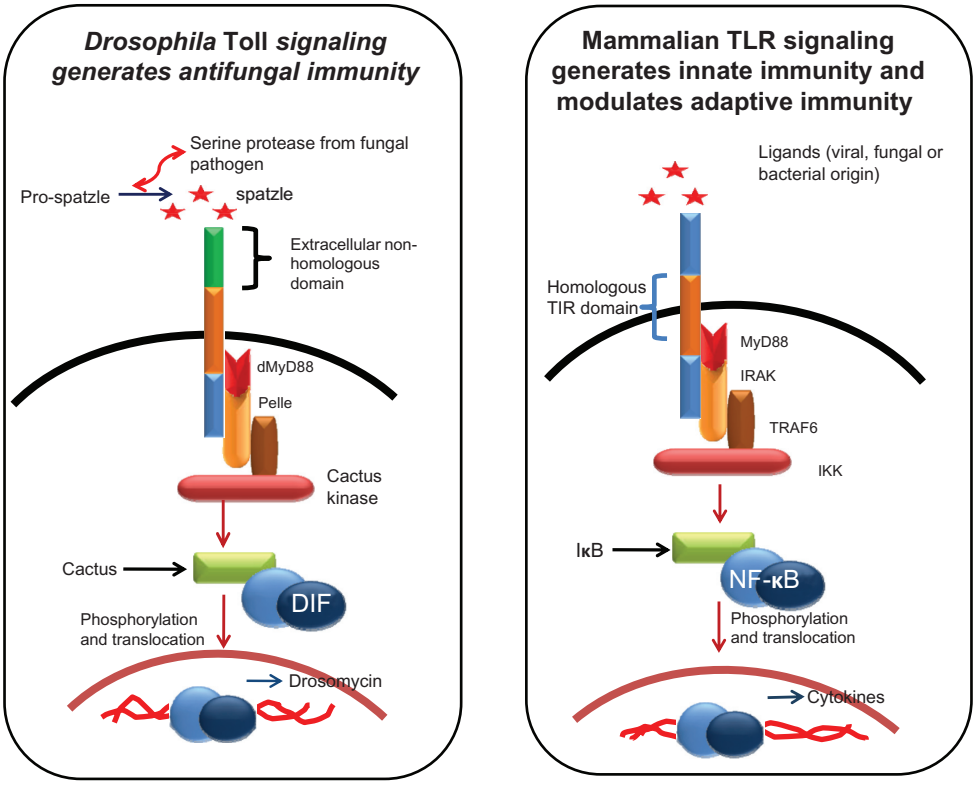

Figure 1. Homology of cell signaling pathways for "Toll" in *Drosophila* and "TLR" in mammalian cells.
Abbreviations: TIR, Toll-interleukin 1 receptor domain; MyD88, Myeloid differentiation primary response gene (88); IRAK, Interleukin-1 receptor associated kinase; TRAF, TNF receptor associated factors; IKK, Inhibitor of NF-κB kinase; IKB, inhibitor of NF-κB; NF-κB, Nuclear factor kappa-light-chain-enhancer of activated B cells; DIF, dorsal related immunity factor.

TLR Response in Innate Immune Cells

Toll-like receptors are known to play a key role in sensing microbial components and inducing innate immune responses. So far, in mammals 13 TLR paralogues (11 are expressed in humans and 12 in mice)[9] have been identified, each responsible for the 'pattern-recognition' of distinct invariant microbial structures and those are widely expressed by all kinds of immune cells, eg, dendritic cells, macrophages, NK cells, T cells, B cells and some non immune cells, eg, fibroblasts, epithelial cells and keratinocytes. Most of the TLRs (1, 2, 4, 5, 6, 10 and 11) are widely expressed on the cell surfaces and other TLRs (3, 7, 8 and 9) are expressed in the endosomal compartments. Presence of a stop codon has made human TLR11 a non-functional one.[10] Functional TLRs are either homodimer (TLR4) or heterodimer ($TLR2^{+}TLR6$) unless there are no mutations or inhibitions. Signaling by the TLRs functions through the TLR signal transduction domains known as Toll/IL-1 receptor domains (TIRs), which can interact with cytoplasmic adaptor proteins including myeloid differentiation primary response gene 88 (MyD88), Toll-interleukin-1 receptor (TIR) domain containing adapter protein (TIRAP)/MyD88-adapter-like (MAL), TIR-domain-containing adapter-inducing interferon-β (TRIF). Signaling pathways activated by TLRs result in the activation of the transcription factor NF-κB, with subsequent production of either pro-inflammatory or anti-inflammatory cytokines. TLR ligands are very diverse in nature and widely implicated to study the different immunological conditions. The following list (Table 1) reveals the names of presently known ligands for the respective TLRs.[11–25] According to their molecular characteristics, TLR ligands can be broadly divided into three categories: lipid (eg, Polyinosinic-polycytidylic acid (poly I:C), nucleic acids (eg, viral ssRNA, CpG motif of bacterial and viral DNA) and protein (eg, viral envelope protein)[10] and have diverse effects on innate immune cells.

Other evidences have demonstrated the presence of some endogenous ligands for TLRs, eg, host-derived nucleic acids and proteins (Table 2).[4,26–43] These results argued the traditional paradigm that major functions of TLRs were considered to distinguish self-non-self but supported the 'Danger-hypothesis': "The immune system has primarily evolved to recognise danger signals rather than non-self signals" by endogenous ligand recognition by TLRs expressed on innate immune cells is one of the major conveners in certain autoimmune disorders by inducing 'sterile' inflammation.[44] During cancer progression, TLRs' endogenous ligands may cause chronic inflammation leading to the T and NK cell dysfunction which is a result of recruitment of myeloid suppressor cells and down-regulation of T-cell and natural killer (NK) cell receptor zeta chain.[45] Emerging evidences also suggest that endogenous ligands of TLRs function not only to induce defensive antimicrobial immune responses but also as a sensitive detection system to initiate tissue regeneration after injury and thereby help in maintaining homeostasis. The mechanisms of endogenous activation of mammalian TLRs may have major implications for the understanding of multiple pathophysiological conditions and disorders.

Several transcription factors other than NF-κB, (eg, AP-1, ELK-1, CREB and STATs) are also known to be activated in different TLR signaling pathways in mammal.[46] TLRs associated different signaling pathways and their biological responses vary widely in different cell types and organs. TLR mediated signaling is involved in DC maturation and activation. Even this signaling is involved in generation of tolerogenic DC. TLR signaling also has great implication in macrophage activation and function. As both cells have great impact in infectious diseases, in generation of anti-tumor immunity and in autoimmune diseases, simultaneously being functional modulators, TLRs play a major role in these conditions.

Although lipopolysaccaride (LPS) works as a potent ligand for TLR4, there are reports suggesting that in different micro environmental context mouse splenic macrophages can respond to an intraperitoneal injection or in vitro treatment of LPS by increasing TLR2 gene expression but not TLR4. Specific inhibitors of mitogen-activated protein kinase (MAPK)/extracellular signal-regulated kinase (ERK)[47] and p38 MAPK could not significantly inhibit TLR2 mRNA upregulation by LPS.[48] In contrast, at high concentration of curcumin, LPS-mediated TLR2 mRNA induction can be abrogated, which might involve curcumin mediated regulation of LPS-induced NF-κB activation.[48] So it is well proven that TLR2, in contrast

Table 1. Exogenous ligands of toll like receptors.

Toll like receptors	Ligands	Function(s)	Ref(s)
TLR1	• Bacterial lipoproteins	• Strong NF-κB responses upon stimulation	• Goethals S, et al[11]
TLR2	• Peptidoglycans • Lipoproteins (MALP-2) • Lipoteichoic acids • Zymosan • Phospholipo mannan (PLM)	• Gram-positive bacterial recognition • PLM-induced response	• Takeuchi O, et al[12] • Jouault T, et al[13]
TLR3	• Viral dsRNA • Polyinosinic-polycytidylic acid (poly I:C)	• Activation of gammadelta T cells in antiviral immunity	• Wesch D, et al[14]
TLR4	• Lipopolysaccharides (LPS) • Viral envelope protein (RSV, MMTV) • Mannans • Mannoproteins	• Release of TNF and other cytokines that ultimately immunity against Gram-negative bacteria • Immunity against activation of macrophage in viral immunity	• Poltorak A, et al[15] • Netea MG, et al[16] • Tada H, et al[17] • Bellocchio S, et al[18]
TLR5	• Flagellin	• Stimulates NF-κB	• Hayashi F, et al[19]
TLR6	• MALP-2 • Zymosan	• Induce Th1 Cytokines (TNF, IFN-γ, IL-12), Th2 cytokines (IL-4) and anti-inflammatory genes (Slpi, IL-10)	• Galanos C, et al[20]
TLR7	• Viral ssRNA • R-848 • Imiquimod • Loxoribine	• Induction of IFN-α, IL-6, and IL-12	• Diebold SS, et al[21]
TLR8	• Imiquimod, eg, 3M-001, -2, and 3 • Loxoribine	• Induce TNF-α, IL-12p40	• Heil F, et al[22]
TLR9	• CpG motifs present in bacterial and viral DNA	• Induce secretion of type I IFN in DC. Induce secretion of IL-6 in B-Cell	• Sun CM, et al[23]
TLR10	• Orphan?	—	• Hasan U, et al[24]
TLR11	• Profilin from *Toxoplasma gondii*	• Parasite-induced IL-12 production	• Yarovinsky F, et al[25]

to TLR4, can be induced in macrophages in response to bacterial infections and may accelerate the innate immune response against pathogens.[48] Downstream signaling molecule, eg, IRAK-4 (involved in TLR2/TLR4 mediated pathway) is also required for the proper maturation of dendritic cells by proper ligand (eg, LPS) stimulation, particularly in terms of cytokine production and the ability to stimulate T helper cell differentiation.[49]

Recently, IRAK-4 was also found to be crucial for type I interferon (IFN) production through TLR7, TLR8 and/or TLR9 in response to viral DNA and RNA.[50] Sequential activation of TLR4 by mmLDL (minimally oxidized LDL) promotes ROS through Syk, PLCγ1, PKC, and gp91phox/Nox2 and simultaneously stimulates expression of proinflammatory cytokines. These observations suggest the mechanisms by which some of the endogenous ligands may induce TLR4-dependent activation of macrophages.[38]

Although TLR10 is an orphan and showing signs to be an interacting partner for TLR2, it can be expressed by only $CD1a^+$ DC subset derived from $CD34^+$ progenitor cells and matured B cells which resemble Langerhans cells in the epidermis. In addition, TLR-signaling induces the upregulation of various maturation markers, such as CD80, CD83 and CD86, and the chemokine receptor CCR7 and both class I/class II MHC.[51] It is reported that TLR genes are generally expressed in different subsets of

Table 2. Endogenous ligands for TLRs.

Ligands	TLRs	Function(s)	Ref(s)
Hsp60	TLR4	• Elicits a potent proinflammatory response	• Ohashi K, et al[26]
Exogenous Hsp27	TLR4	• As potent as LPS. • Down regulate TLR4 as Hsp27-TLR4 complex internalize.	• Yusuf N, et al[27]
Hsp22 (HSPB8)	TLR4	• TLR4 dependent activation of dendritic cell.	• Roelofs MF, et al[28]
MRP8/S100A8	TLR4	• Activate TLR4 signal.	• Vogl T, et al[29]
MRP14/S100A9	TLR4	• Activate TLR4 signal.	• Vogl T, et al[29]
HMGB1 (high mobility group box 1)	TLR2/TLR4/TLR9	• Differential usage of TLR2 and TLR4 in primary cells and in established cell lines. • DNA, which is released into the systemic circulation after traumatic shock or injury, presented to TLR9.	• Yu M, et al[30] • Tian J, et al[31]
SAA (Serum amyloid A)	TLR2/TLR4	• Induce inflammation.	• Cheng N, et al[136] • Sandri S, et al[33]
Extradomain A of fibronectin	TLR4	• Inflammatory response.	• Okamura Y, et al[34]
αA crystallin	TLR4	• DC maturation. • Cytokine production.	• Roelofs MF, et al[28]
GP96	TLR2/TLR4	• Mobilise NF-κb, • Activate mitogen-activated protein kinase. • Induce dendritic cell maturation. • Cytokine synthesis.	• Zhang Z, et al[35]
Fibrinogen	TLR4	• Induce the production of chemokines from macrophages through TLR4.	• Smiley ST, et al[36]
Lung surfactant protein A	TLR4	• Induction of the activation of the NF-κB signaling pathway and up-regulation of cytokine synthesis.	• Zhang Z, et al[35]
TOLLIP, PPAR and TIR8	?	• Negatively regulate TLR signaling pathways suppression of inflammatory responses.	• Cario E, et al[37]
mmLDL (minimally oxidized low-density lipoprotein)	CD14/TLR2/TLR4	• Mediated ROS generation.	• Bae YS, et al[38]
Chromatin–IgG complexes multiple	TLR9	• B-cell activation.	• Wagner H[39]
Heparan sulphate	TLR4	• Maturation of dendritic cell.	• Johnson GB, et al[40]
Oligosaccharide of hyaluronan (soluble hyaluronan)	TLR4	• Activation of DC.	• Termeer C, et al[41]
β-defensin 2-lymphoma antigen idiotype sFv fusion protein	TLR4	• Activation of DC	• Biragyn A, et al[4]
siRNA/shRNA	TLR3	• Activation of IL-8, and TNF-α; activation of NF-κB promoters.	• Kariko K, et al[42]
Immune complexes containing self RNA or DNA, self proteins	TLR7 or TLR9	• Plasmacytoid dendritic cells (pDCs) become activated • Secrete IFN-γ.	• Krieg AM[43]

innate immune cells, eg, human TLR7 and TLR9 are exclusively expressed in plasmacytoid DCs. In contrast, TLRs 2, 3, 4, 5 and 8 are expressed in myeloid DCs.[52] If simultaneously, two TLRs are activated on DCs that may lead to prolonged activation of various MAPK and transcription factors with increased production of pro-inflammatory cytokines.[52] Studies have shown that activation of IFN-regulatory factor (IRF) that is mediated by TLRs can directly regulate the IL-12 transcription.[53] Sometimes, combinatorial activation of multiple TLRs can act either synergistically (eg, TLR3 plus TLR7) or antagonistically (eg, TLRs 7, 8, 9 plus TLR2).[54] TLR2 ligands, generally promote either Th2 or Treg response and any defect in TLR2 mediated signaling generally lead to higher proinflammatory response with Th1 polarization.[55] However, LPS stimulated TLR4 in DCs has been suggested to induce potent p38 and JNK1/2MAPK activation which leads to the induction of interleukin-12p70 which elicits Th1 response.[51]

Zymosan mediated TLR2 signaling has been reported to activate both suppressor of cytokine signaling 3 (SOCS3) and SOCS1 in tolerogenic DCs via IL-10 and retinoic acid secretion and in turn skew the Th1 or Th17 response.[56] Ligand mediated activation of TLR2/1 and TLR4 leads to SOCS1 induction. Even SOCS1-deficient macrophages produce greater amounts of IL-12 in response to TLR signal activation. One of the critical negative signaling regulators of TLR-signaling, PI3K is effective to modulate the magnitude of the immune responses to pathogens.[57] Macrophages and DCs from PI3K knockout mice can produce more IL-12 and modulate the Th1/Th2 balance than wild type DCs in response to various TLR ligands.[58] PI3K-dependent activation of the mammalian target of rapamycin (mTOR) signaling can promote the production of type I IFNs by TLR ligands or virus stimulated pDCs.[59,60]

Modulation of Adaptive Immunity by TLR Response

TLRs have been reported to function as evolutionary conserved pattern recognition receptor (PRR).[44] Moreover, TLRs are traditionally known to be one of the most efficient modulators of innate immunity. However, recent evidences possess a challenge to this concept and lead to a slow but steady change in notion suggesting an important role of TLRs to modulate adaptive immune response. It has been suggested by different groups that association of differential TLR response to modulate adaptive immunity especially specific T cell response like $CD4^+$ T cell specific Th1, Th2, Th17 and also $CD8^+$ T cell associated altered CTL response.[51] Moreover, B cell mediated humoral immunity is also found to be modulated by specific TLR ligation.[61] Presence of TLR ligands can activate the maturation of immature DCs and that leads to secretion of a variety of cytokines and enhanced surface expression of costimulatory molecules. These matured and activated DCs then start presenting antigen to T cells which simultaneously leads to antigen specific T cell expansion. This event helps in $CD4^+$ T cells differentiation into helper T (Th) cells, Th1, Th2 or Th17 cells. Th1 cells then can secrete IFN-γ, IL-2 to drive away invading bacteria or viruses. Th2 cells secrete IL-4, IL-10 or IL-13 and are involved in modulation of humoral immunity in different diseased conditions. Th17 cells preferentially produce IL-17A, IL-17F, IL-21 and IL-22.[62] Th17 responses, when uncontrolled, result in many human autoimmune diseases, such as, rheumatoid arthritis (RA), multiple sclerosis (MS), inflammatory bowel disease (IBD), psoriasis and uveitis.[62] It has been shown that IL-23 plays a pivotal role in expansion of Th17 cells.[62] In $TLR2^{-/-}$ mice, expression of IL-23 was found to be higher and DCs isolated from $TLR2^{-/-}$ mice produced significantly higher IL-23 compared to normal mice.[56] In experimental autoimmune encephalomyelitis (EAE) model, stimulation with TLR2 agonists promoted Th17 differentiation in vitro and resulted in robust proliferation and IL-17 cytokine production. TLR2 regulates Th17 cell-mediated autoimmunity in vivo and loss of TLR2 in $CD4^+$ T cells dramatically ameliorated EAE. This study thus reveals an important role of TLR in the direct regulation of adaptive immune response and pathogenesis of autoimmune diseases.[63] TLR signaling, most of the times, activates DCs which can produce Th1 inducing cytokines, such as, IL-12 or IL-18, thereby promoting Th1 polarization.[10] Microbial stimulation of TLRs in monocytes, macrophages and dendritic cells initiates type-1 cytokine pathway and that triggers the production of cytokines, eg, IL-12 and IL-23 that activate lymphocytes to produce IFN-γ.[64] Certain TLR ligands are known to induce production of IL-10 which stimulates Th2 or T regulatory responses.[65]

Ligands for TLR4, 9, 3 and 7 can generate Th1 response by activating JNK and p38 and also induce production of IL-12p70.[66] On the contrary, TLR2 ligands, can activate either a Th1 or a Th2 or a T-regulatory response in different cellular context through induction of IL-10 and TGF-β by DCs.[12] ERK appears as one of the determining signaling molecules involved in Th2 polarization and regulation of autoimmunity in a TLR2 dependent manner.[67] In addition, TLR ligands mediated stimulation directly on mice B cells can effectively induce robust antibody responses.[68] In vitro stimulated murine B cells by TLR4 and TLR9 ligands can effectively proliferate and secrete antibody.[69] It has been reported that polyclonal expansion and differentiation of mouse naïve B cells is independent of TLR5 and 8 ligands mediated signaling.[61] Moreover, recent studies also described that TLRs on B cells play a critical role in autoantibody production.[70] TLR9 ligand, CpG can efficiently modulate the kinetics, efficacy and self life of the memory B-cell response.[71]

TLR Response in T Cells

Immune response and its regulation involve complex cellular response, where T cells signify adaptive immunity in collaboration with antigen presenting cells.[72–75] Recently TLRs have been suggested to be expressed in T cells and have future implication in host immunity including tumor immunity.[47,51,76–83]

Toll-like receptors (TLRs) function as the sensor of pathogen pattern recognition molecules and initiate innate and adaptive immune responses during microbial infections, cancer and autoimmunity and subsequently tunes the adaptive immunity. Recognition of pathogen-derived ligands by TLRs are expressed on different types of cells, including dendritic cells, macrophages and T cells, triggers the NF-κB and type-1 interferon pathways, leading to the activation of respective cell types and initiates the production of proinflammatory cytokines that are essential in stimulating $CD4^+$ T cells to differentiate into T helper (Th1, Th2, Th17) and regulatory T (Treg) cells. It has been reported that human TLR2, 3, 4, 5 and 9 proteins are expressed intracellularly in stimulated T cells.[78] So there may be differential expression profile of all these TLRs in activated T cell subsets and which may be functionally relevant. Some TLR ligands may promote $CD4^+$ T cell survival.[84] TLRs have the ability to function as one of the major co-stimulatory molecules to either activate or suppress different T cell subsets ($CD4^+$ and $CD8^+$ T cells).[82] Both murine and human $CD4^+$ T cell functions have been suggested to be regulated by TLR2.[32] TLR2 also acts as co-stimulatory signaling receptors in activated $CD8^+$ T cells for proliferation and survival through the enhanced expression of CD25 and Bcl-xL.[85] TLR2 engagement on $CD8^+$ T cells lowers the threshold for optimal antigen-induced T cell activation.[86] There are evidences which have shown the direct role of TLRs on Treg's suppressing activity.[87] Treg cells' suppressive activity depends on mostly contact-dependent mechanisms and also on the secretion of suppressor cytokines (eg, IL-10, TGF-β, IL-35, etc).[88] Enhanced Treg suppressive activity have been proposed to depend on functional TLR4[76] or TLR5,[89] while TLR2 ligation on mouse or TLR8 ligation on human Treg cells have been suggested to reverse Treg suppressive function. Luciferase reporter assays in Jurkat T cell line demonstrated that TLR10 gene expression may be transcriptionally controlled by FOXP3 indicating that TLR10 may have a contribution in immune-suppressive function of Tregs.[90] It has been shown in recent studies that the human TLR8 signaling pathway is essential for reversing the suppressive functions of Treg cells which play a critical role in suppressing immune responses and in inducing immune tolerance in cancer and infectious diseases.[91] On the contrary, TLR2 expressed on the Treg may help to increase their survival but does not affect their suppressing activity.[32] TLR2 signaling stimulates DCs which in turn activates retinoic acid metabolizing enzyme retinaldehyde dehydrogenase type 2 and suppresses Th17- and Th1-mediated autoimmune responses.[56] TLR2 induction in DC regulates p38 activation by activating SOCS3 and simultaneously mediates production of IL-10 by activated ERK. These results suggest that TLR2 signaling may be one of the major contributors of Treg development and proliferation.[56] It has been reported that TLR4 induction involves activation of p38 and JNK MAPK signaling, which may induce Th1 response and simultaneous production of proinflammatory cytokines, IFN-γ, TNF-α and IL-6.[92,93] Linking TLR signaling to control Treg cells opens enormous opportunities to manipulate TLR signaling to control both innate and adaptive immunity against cancer and infectious diseases. Human γδ T cells express TLR3 which may act

as co-stimulatory effectors with the aid of the ligand polyinosinic-polycytidylic acid (poly I:C) on TCR-stimulated IFN-γ production.[94] Diverse TLR (2–9) agonists, despite their operation through common pathways, induce distinct cytokine/chemokine profiles that in turn have little or no overlap with TCR-mediated response.[95] TLR3 ligand has been reported to be involved in autoimmunity by directly inducing the synthesis of IL-17A and IL-21 and drive differentiation of human naive $CD4^+$ T cells.[96] Even TLR-stimulated dendritic cells may induce specific T cells to differentiate into memory cells.[97]

Anti-CD3/CD28 activation on $CD4^+$ T cells has been demonstrated to maintain TLR5 and 9 mRNA levels but has no effect on TLR2.[31] Moreover, it has been shown that there is an upregulation of cell surface TLR4/MD2 expression on an emigrating subset of dendritic epidermal T cells in a LPS independent manner.[32] Even in the absence of antigen-presenting cells (APCs), TLR5 and TLR7 and/or TLR8 ligands may act synergistically to stimulate human memory $CD4^+$ T cells at the suboptimal level, both TCR-dependent or TCR-independent manner to enhance the proliferation and production of IFN-γ, IL-8 and IL-10.[98]

Recently it has been shown that Porin of *Shigella dysenteriae* type 1 might be involved in upregulation of TLR2 on anti-CD3-stimulated $CD4^+$ T cells but fails to elicit the expression of other TLRs. The role of porin in T cell proliferation is supported by the fact that it induces the expression of IL-2 and CD25. It is also known to trigger the effector function of T cell, which is evident from MyD88-dependent release of type 1 cytokines, tumor necrosis factor (TNF) and IFN-γ along with the induction of type 1 chemokines, macrophage inflammatory protein-1 (MIP-1) and MIP-1 and their receptor, CCR5, which testifies the ability of porin to activate the adaptive immunity.[99]

TLR2 may provide co-stimulatory signal in activated $CD8^+$ T cells which results in higher proliferation, survival and enhanced expression of CD25 and Bcl-xL.[85] It has been documented that TLR signaling helps in maintaining T cell memory in humans with enhanced expression of memory markers. Without any help from APCs, flagellin (a TLR5 ligand) and R-848 (a TLR7 and/or TLR8 ligand) can conduct both suboptimal TCR-dependent or TCR-independent activation and enhancement of proliferation with enhanced production of IFN-γ, IL-8 and IL-10 by human $CD4^+$ T cells.[82,98]

It has been established that T cell development in IRAK4-deficient mice is unaffected while proliferation is severely impaired. So, it appears that during thymic selection, TCR-mediated NF-κB activation via IRAK4 is not important or may be that vigorous NF-κB activation via IRAK4 mediated pathway is not needed for T cell development which needs only weak stimulus. It has been established that IRAK4-deficient T cells can be stimulated with soluble anti-CD3 antibody but their stimulation is marginally impaired when stimulant is immobilized plate-bound anti-CD3 antibody.[49] There is evidence that Carma1-Bcl-10-mucosa associated lymphoid tissue 1 (Malt1) (CBM) complex promotes TCR-mediated NF-κB activation.[100–105] Any deletion or mutation in CBM can completely perturb this process as well as TRAF6 mediated signal in T cells.[106] TRAF6 is another signaling molecule involved in downstream of the CBM complex signaling in T cells as well as TLR signaling pathway in any immune cells.[107] In T cell, IRAK4-ZAP-70 complex activates PKCθ, which may in turn, activates the CBM complex. In the context of TCR-induced NF-κB activation, far positional difference between IRAK4 and TRAF6 in the IRAK4 mediated signaling cascade proves that IRAK4 cannot directly activate TRAF6. However, there is no such direct indication like MyD88-IRAK4 complex in T cell which may mediate NF-κB activation via TRAF-6. There may be a possibility that over-activation of IRAK4 pathway in T cell following both TCR induction and TLR-ligand association promotes either activation induced cell death (AICD) or anergy.

Collectively, these studies demonstrated some microbial components and even some endogenous TLR ligands may stimulate TLR responses in T cells and those either enhance T cell proliferation, cytokine and chemokine productions and effector functions by T cells or regulate its suppressive functions (Fig. 2). As both TLR and TCR-induced NF-κB activation in T cells is IRAK4-dependent, there may be a cross-talk between these two signaling pathways in T cells.[49,108–110]

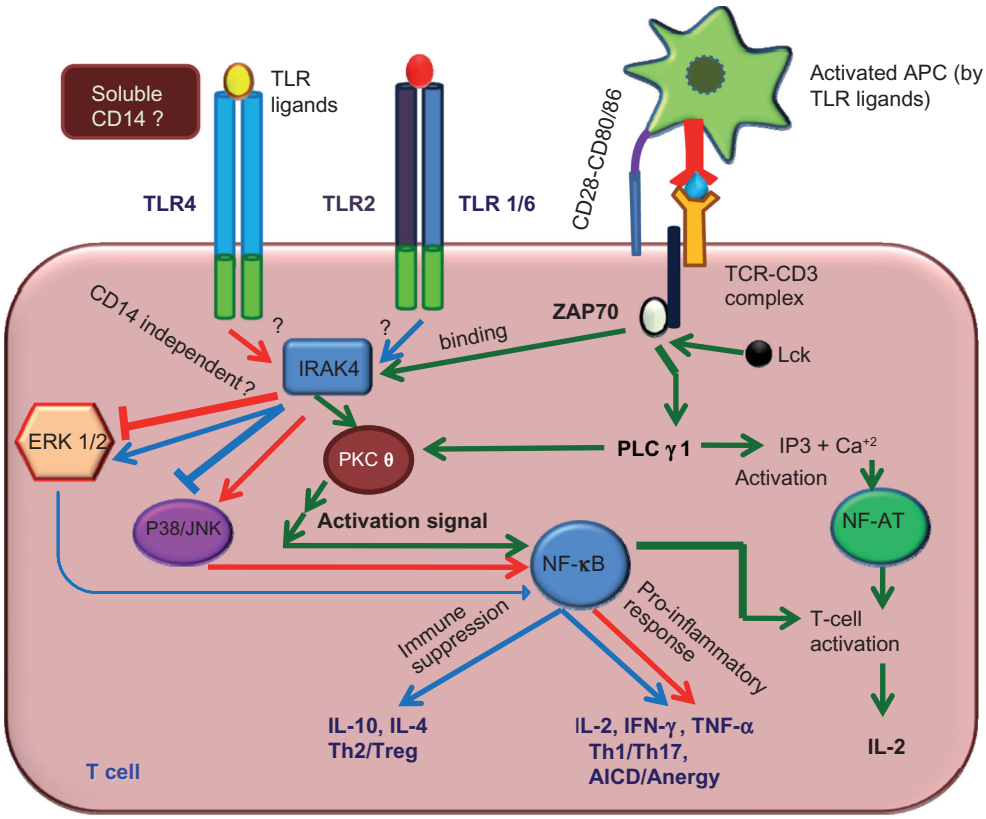

Figure 2. Proposed signaling events of TLR response in T cell: T cell responds through TCR-MHC interaction while interacting with APCs subsequently through the activation of several signaling molecules, like ZAP 70, Lck, PKCθ etc. T cell activation can lead to IL-2 production.[132,133] Moreover, it can also lead to IRAK-4 mediated downstream signaling cascades which involve NF-κB activation while NFAT might be unaffected by this signaling event. IRAK4 mediated signaling events might differentially function during TCR stimulation and TLR driven innate immune pathway.[49,108–110,134] Additionally, differential MAPK signals may polarize respective T cell response (eg, Th1 and Th2 or Treg).[56,79,135] There may be several yet unknown issues like CD14 dependent and independent TLR response in T cells, which needs to be explored in future. Blue, red and green lines are proposed for TLR2, TLR4 and TCR mediated response(s) respectively, where possible interactions might be operative for putative TLR response in T cells.

TLRs in Modulating Immunity and Its Implication in Immunotherapy

Recently, TLRs have been proposed to play an important role in translational research. There are several reports which suggest the potential implication of TLRs in immunotherapy and clinical investigations. It has been shown that activation of TLR5 by its ligand flagellin was able to inhibit cell proliferation in breast cancer.[111] In contrast, it was also found to promote proliferation of cells in gastric cancer.[112] Chemotherapy or total body irradiation (TBI) and lymphodepletion before adoptive transfer of tumor-specific T cells is a critical advancement in the treatment of patients with melanoma. More than 50% of patients that are refractory to other treatments experience an objective or curative response with this approach.[113]

Combined immunization of hCG-beta along with TLR agonists (poly ICLC, resiquimod) has been shown to elicit greater immune responses that in turn results in better clinical benefit. Activation of APCs coupled with TLR agonists enhances the efficacy of cancer immunotherapy.[114] Unmethylated cytosine-phosphate-guanine (CpG) dinucleotides in microbial DNA activate TLR9. CpG oligonucleotides can induce Th1 and Treg type cytokines and suppress the Th2 response.[115] It has been shown that a two-component mRNA-based tumor vaccine drives the Th1 response mediated by TLR7.[116] It has been demonstrated that activation of TLR-MyD88 signals in patient-derived T cells reduced the activation threshold to tumor antigens resulting in increased cytokine production, expansion and cytotoxicity.[117] TLR7/8 agonists have long aroused interest because they not only activate the APCs but also activate T and NK cells. Gardiquimod and imiquimod promote activation of splenic T, NK and NKT cells in mouse. Simultaneously, enhanced

expression of co-stimulatory molecules and IL-12 by macrophages and bone marrow DCs have been observed. TLR7/8 agonists can act as potent response modifiers in tumor therapy.[118] Lentiviral activation of DC was TLR dependent, as it has been shown to be inhibited by TRIF/MyD88 knockout. Moreover, lentivirally transduced DC lacking TLR3 or TLR7 had an impaired capacity to induce antigen specific $CD8^+$ T cell response.[119] Also in cancer immunotherapy TLR2, -3 and -9 have been shown to be directly involved in apoptosis.[120] Synergistic CD40/TLR activation also induced the migration of activated dendritic cells to lymphatic locations and promoted their capacity to present antigens. Correspondingly, without exogenous antigen, combined CD40/TLR agonists boosted measurable T-cell-mediated antitumor immunity and induced the rejection of otherwise lethal ovarian carcinomas.[121]

Inflammatory mediators and especially the TLR family of proteins have been shown to play a pivotal role in inducing the immune activation program in DCs. TLRs recognize pathogen-associated-molecular-patterns (PAMPS) like LPS or flagellin and signal to alert immune cells in general, and DC in particular. DC activation, also referred to as DC maturation, thus results in immunity.[122] Intra-tumoral injection of Pam3Cys-SK4 (TLR1/2 agonist) or R848 (TLR7 agonist) also produced a significant survival benefit in glioma-bearing C57BL/6 mice.[123] Thus it has been observed that there is increased frequencies of tumor-infiltrating IFN-gamma producing $CD4^+$ and $CD8^+$ effector T cells.[123] Pre-treatment of DCs with TLR ligands resulted in the triggering of many more TCRs in responding $CD8^+$ T cells.[124] Accordingly, DCs may provide a functional link between innate and acquired immunity. Also plasmacytoid DCs express different sets of TLRs that recognize a broad range of conserved molecular patterns of pathogens.[125]

In appropriate activation of TLRs by self-components can result in sterile inflammation or autoimmunity.[126] Self-recognition by TLR7 and TLR9 has been reviewed as an important part of the development of lupus and other autoimmune diseases.[126] Immunotherapy may be improved by using TLR synergy to enhance the parasite-specific immune response.[127] Several other agonists of TLRs 4, 7, 8 and 9 were also shown to be effective for treatments of infections and cancers and, furthermore, were used as adjuvants for vaccination.[128]

Specific TLRs response (using TLR ligands and modulation of TLR signaling) to regulate respective disease scenario or partly the regulatory process of disease patho-biology with the TLR driven immune-modulation may have the possibilities for challenging scope of future immunotherapy.[111–113,129]

Future Direction and Yet Unanswered Issues for TLR Response in T Cells

It is quite interesting that TLR which was extended from homology of insect developmental regulatory protein and then incorporated in mammalian innate immune response as a sensing receptor, now became one of the critical players in modulating adaptive immunity. In very recent times, it has been demonstrated that TLRs are also expressed in T cells and TLR ligands may alter specific T cell response. However, TLR response in T cells, especially more in-depth knowledge is warranted for the future immuno-biological investigations. There are few issues which are not clear till date to dissect out the "TLR response in T cells".

- Whether a subset of T cells which express specific TLRs can functionally be distinct from other T cells which may not express the respective TLRs? For example, it is not clear whether effector T cells, regulatory T cells and memory T cells express distinct functional TLRs for the specific T cell phenotype and function?
- Whether TLR ligands are associated with bystander or antigen specific T cell function?
- For certain TLRs, eg, TLR4 response in T cells, where CD14 is an important co-receptor for TLR4 signaling may be limited.[130,131] So, it is possible that T cell may execute CD14 independent TLR4 response.
- For a T cell–antigen presenting cell (APC) cross-talk there may be both direct and indirect effect of TLRs for directing T cells for an ongoing immune response for the respective T cell polarization (eg, Th1, Th2, Th17, Tc1, Tc2, Treg response).

- Are there any structural and functional differences exist for the respective TLRs in case of specific T cells and innate immune cells as interacting APCs?
- Although role of TLR is just started with immune-modulatory effect in T cells, it is not clear whether TLR specific T cells can equally work in translational research like immunotherapy and specific vaccination strategies.
- Moreover, it will be also interesting to explore whether TLR also sense antigen specificity and could be designated as one of the new members of immune receptors in bridging the innate and adaptive immunity.

Abbreviations

AP-1, Activator protein 1; CCR5, C-C chemokine receptor type 5; CBM, Carma1–Bcl-10–mucosa associated lymphoid tissue 1 (Malt1); CMI, Cell mediated immunity; CREB, cAMP response element-binding; DC, Dendritic cell; ELK, ETS LiKe gene; ERK, Extracellular signal-regulated kinase; GM-CSF, Granulocyte-Macrophage Colony Stimulating Factor; HMGB, High mobility group box; IL, Interleukin; IFN, Interferon; IRAK, Interleukin-1 receptor associated kinase; IRF, IFN-regulatory factor LPS, Lipopolysaccarides; MAL, MyD88-adapter-like; MALP, Macrophage-activating lipopeptide; MAPK, Mitogen-activated protein kinase; MIP, Macrophage-inflammatory protein; MHC, Major histocompatibility complex; mmLDL, minimally oxidized LDL; MMTV, Mouse mammary tumor virus; MRP, Myeloid-related protein; mTOR, mammalian target of rapamycin; MyD88, Myeloid differentiation primary response gene (88); NF-κB, Nuclear factor kappa-light-chain-enhancer of activated B cells; NOX2, NADPH oxidase; pDC, Plasmacytoid dendritic cells; PKC, Phospho kinase C; PLCκ1, Phospholipase C gamma 1; PPAR, Peroxisome proliferator-activated receptor; RSV, Respiratory Syncytial Virus; SAA, Serum amyloid A; SOCS, Suppressor of cytokine Signaling Proteins; STAT, Signal Transducers and Activators of Transcription; Syk, Spleen tyrosine kinase; TCR, T cell receptor; TIRAP, Toll-interleukin 1 receptor (TIR) domain-containing adapter protein; TLR, Toll like receptor; TOLLIP, Toll interacting protein; TRIF, TIR-domain-containing adapter-inducing interferon-β (TRIF).

Acknowledgements

The work was supported by Department of Biotechnology, Ministry of Science and Technology, Govt. of India (reference no: BT/PR13312/GBD/27/247/2009).

Disclosures

Author(s) have provided signed confirmations to the publisher of their compliance with all applicable legal and ethical obligations in respect to declaration of conflicts of interest, funding, authorship and contributorship, and compliance with ethical requirements in respect to treatment of human and animal test subjects. If this article contains identifiable human subject(s) author(s) were required to supply signed patient consent prior to publication. Author(s) have confirmed that the published article is unique and not under consideration nor published by any other publication and that they have consent to reproduce any copyrighted material. The peer reviewers declared no conflicts of interest.

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