Arch Pharm Res Vol 32, No 11, 1485-1502, 2009
DOI 10.1007/s12272-009-2100-6

Archives of Pharmacal Research
www.springer.com/12272

REVIEW

Drugs Targeting Toll-like Receptors

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(Received July 29, 2009/Revised September 17, 2009/Accepted September 24, 2009)

Animals and plants are exposed to myriads of potential microbial invaders. In case of animals, Toll-like receptors (TLRs) act as the primary defense against infection by pathogens. Arguably, less is known regarding the activation of TLRs that connect the innate and adaptive immune systems. Some TLR ligands have been used as adjuvants in various vaccines and have gained a great deal of attention due to their ability to elicit an effective immune response. Understanding the intricate relationships between various molecules involved in TLR signaling and their positive or negative regulation is a key focus for the development of effective therapeutics. In this review, recent developments in TLR signaling that will be very important in providing new drug target molecules and a better understanding of molecular regulation of innate immunity are discussed.

Key words: Agonist, Drug, Inhibitor, Innate immunity, Toll-like receptor

INTRODUCTION

Toll-like receptors (TLRs) are the prime pathogen sensing gates in the body. TLRs play essential roles in the innate immune responses to microbial pathogens based on their ability to recognize pathogen-associated molecular patterns (PAMPs) (Akira et al., 2006; Krishnan et al., 2007). TLRs1-9 are conserved between humans and mice. In addition, TLR10 is expressed in humans but not in mice, whereas TLR11 is present in mice but not in humans. TLRs1, 2, 4, 5 and 6 are primarily located on the cell surface and recognize bacterial components. TLRs3, 7, 8 and 9 are generally located in the endocytic compartments and primarily recognize viral products (Lee and Kim, 2007). Specifically, TLR4 captures lipopolysaccharide (LPS) in association with MD2 which enhances TLR4 signaling. TLR1/2 and TLR1/6 heterodimers bind with lipopeptides and lipoproteins. TLRs that recognize nucleic acids such as TLR3 (double strand RNA; dsRNA), TLR7 (single strand RNA; ssRNA), TLR8 (ssRNA) and TLR9 (dsDNA) are located in the intracellular endosomes. When the transmembrane and cytoplasmic regions of TLR9 are replaced by that of TLR4, this chimeric protein is expressed on the cell surface, after which the cells respond to self DNA, which suggests that the endosomal location of TLR9 is important for the prevention of an immune response to self DNA (Barton et al., 2006).

The activation of intracellular signaling is accomplished by the recruitment of various adaptor proteins that contain Toll/IL-1 receptor (TIR) domain by TLRs. Myeloid differentiation primary response gene 88 (MyD88), TIR domain-containing adaptor protein (TIRAP)/MyD88-adaptor-like (MAL), TIR domain-containing adaptor inducing IFN-β (TRIF)/TIR-domain containing adaptor molecule (TICAM)-1 and TRIF-related adaptor molecule (TRAM) are the adaptors used by TLRs. All TLRs use MyD88 except TLR3 to induce the expression of inflammatory cytokine genes (Medzhitov et al., 1998). TLR2 and TLR4 induced MyD88-dependent signaling requires additional adaptor proteins such as TIRAP/MAL, which bridges TLR2/TLR4 and MyD88 (Horng et al., 2002). In addition, TLR4 uses a MyD88-independent signaling pathway which requires the use of TRIF, the prime adaptor in TLR3 signaling (Yamamoto et al., 2002). TLR3 responds to stimulation with polyinosinic-polycytidylic acid (poly I:C), a synthetic dsRNA analogue, by using TRIF,

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which indirectly activates several transcription factors, including IRF3, NF-κB and AP1 (Yamamoto et al., 2003a). IFNs are produced in response to these stimuli and are often crucial for the induction of effective immunity (Boehm et al., 1997; Stark et al., 1998). TRAM is a specific adaptor molecule in TLR4 signaling and transmits its signaling to TRIF (Yamamoto et al., 2003b). TLRs activate intracellular signaling via interaction with these adaptor molecules containing the TIR domain and induce the release of various inflammatory mediators.

MyD88 interacts with the kinase interleukin 1 receptor-associated kinase 4 (IRAK4), which activates IRAK1 and IRAK2 (Kawagoe et al., 2008). IRAK4 and IRAK1 are subsequently phosphorylated upon stimulation and result in the activation of TRAF6 (TNF-receptor-associated factor 6). TRAF6 is an E3 ubiquitin ligase, that catalyses the formation of Lys-63-linked polyubiquitination on TRAF6 itself and IKK-γ (also called as NEMO) (Deng et al., 2000). This ubiquitination leads to the recruitment of TAK1 (TGF-beta activated kinase 1), TAB2 (TAK1-associated binding protein 2) and TAB3 (TAK1-associated binding protein 3) (Kanayama et al., 2004). The recruited TAK1 in turn leads to the activation of IKK (inhibitor of kappa-B kinase) complex, causing NF-κB activation, which is also enhanced by IRF5 (interferon regulatory factor 5) activated by TRAF6. Furthermore, viral infection causes the formation of MyD88-IRAK4-IRAK1-TRAF6-IRF7 complex (Kawai et al., 2004), where IRF7 is phosphorylated by IRAK1 and/or IKK-α (Uematsu et al., 2005). TLRs induce inflammatory gene expression by regulating the activities of signal-dependent transcription factors that include members of the NF-κB, AP1 and IRF families (Kawai and Akira, 2007; Zhu et al., 2006). This results in the release of a variety of inflammatory mediators, such as nitric oxide, prostaglandins, tumor necrosis factor-α (TNF-α), interleukin (IL)-6, and IL-1β, type 1 interferon (IFN) and chemokines (Beutler, 2004). The

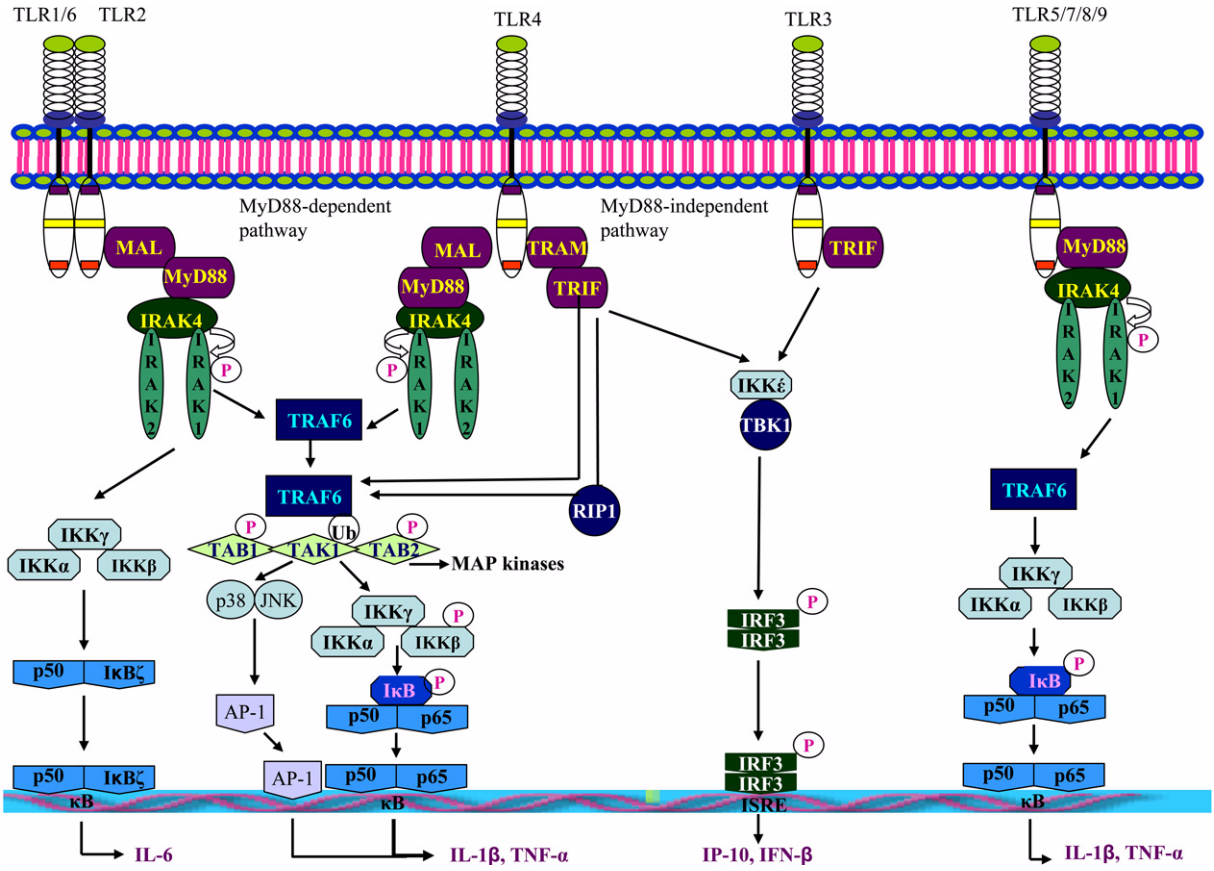

Fig. 1. TLR signaling mechanisms: Adapted from Krishnan et al. (Krishnan et al., 2007). Signaling mediated by TLR is broadly classified as MyD88-dependent and independent pathways. All TLRs utilize MyD88 with the exception of TLR3. MyD88 binds with the TIR domain of the receptor and phosphorylates IRAK4 which in turn phosphorylates IRAK1. IRAK1 phosphorylates TRAF6 leading to the ubiquitination of TAK complex. Activation of IKK, JNK and p38 pathways leads inflammatory and antiviral responses. ISRE, interferon stimulatory response element; κB, κB site; p, phosphorylation; ub, ubiquitination.

simplified diagram of TLR signaling is shown in Fig. 1.

WHY TLRs ARE TARGETED FOR DRUGS

In the recent past, several biotechnology and pharmaceutical companies have been actively engaged in the research on TLR drugs that are either agonists to rectify inadequate immune reaction or antagonists to inhibit over activation. TLRs are double-edged swords which play dual roles as a physiological and pathological mediator, and the dysfunction of TLRs has been implicated in wide range of human diseases including infectious diseases, immunodeficiency, sepsis syndromes, autoimmune disorders, atherosclerosis, malignancy and asthma. More recently, genetic loss-of-function experiments in mice have shown that TLRs contribute to the pathogenesis of a number of diseases in which inflammation is known to play a pathogenic role (Atkinson, 2008). This evidence suggests that TLRs can be targeted for drug therapy at each and every level of their activation. For example, inhibitors of MAPK-ERK and PI3K/AKT pathways, but not that of IFN signaling pathway, were able to block the effect of LPS (Zhang et al., 2009). Moreover, TLR polymorphisms are reportedly a primary factor in various infectious diseases (Schroder and Schumann, 2005). Apart from that, TLR agonists and antagonists can be directly targeted as possible drug candidates. For instance, certain CpG-ODNs act as antagonists of TLR7 and 9 (Yu et al., 2009). Some inhibitors of TLRs and their signaling target molecules are summarized in Table I.

Table I. TLR signaling components and their negative regulators

TLR signaling components	Negative regulators
IRAK1	A20, IRAKM, TOLLIP
IRF3	PIN1
IRF5	IRF4
IκB-α	TRAIL
MAL	A52R, SOCS1
MAL/MyD88	ST2
MyD88	A52R, MyD88s
p50/p50	BCL3, NF-κBID (IκBNS)
p65/p50	PIAS, PIN1
REL	ATF3
TAB1/TAB2	TRIM30-α
TBK1	DUBA, SHP2
TLR2	STLRs
TLR4	RP105
TRAF6	CYLD
TRAM	A52R
TRIF	A52R, SARM, TAG

INHIBITORS OF TLR SIGNALING AS POSSIBLE DRUG CANDIDATES

Recently, there has been growing interest in the mechanisms that directly regulate TLR transcriptional programs. As a result, several new regulatory components have been identified and some of the known components have been assigned new roles. Overactivation of TLRs demands tight regulation. In the following section, we will discuss the negative regulators of the TLR signaling molecules, since such proteins form the hub of potential drugs. In general, degradation of the proteins involved in amplification of inflammatory responses or transcription factors is one of the principal mechanisms that terminate the activation of signaling pathways. This type of regulatory mechanism may occur as a result of TLR activation as short- and long-term responses, directly and indirectly, or as a feedback mechanism. In addition to degradation, there are two important mechanisms by which TLRs are regulated: ubiquitination and competition for binding. Ubiquitination plays an important role in innate immunity by tagging proteins for degradation and is used as a fundamental mechanism of regulation of signal transduction via innate immunity. Though this is a general mechanism of inhibition of innate immunity, there exists some specific mechanisms which depend on the ligands that trigger the signal, and such specificity underpins the sequestration, competitive binding, splice variants, proteins secreted by viruses, agents that stabilize the repressive homodimers, and proteins that directly interact with chromatin. In the following section of the article we will discuss such general and specific mechanisms by accounting the individual characteristics of TLR signaling designed as receptors, adaptors, signaling pathways and transcription factors.

INHIBITORS OF TLRs

Since most TLRs are expressed on the surface, it is possible to block their expression by soluble monoclonal antibodies. Soluble forms of TLR2 (sTLR2) are found in human breast milk and plasma (LeBouder et al., 2003). To date, six sTLR2 polypeptides have been identified in human milk, plasma and monocyte supernatants. Among these, sTLR2 with a molecular weight of 66-kDa is the primary soluble TLR2 polypeptide released by blood monocytes. The reduced production of IL-1 and TNF-α is associated with the interaction between sTLR2 and soluble CD14 complex

in response to bacterial lipopeptide (LeBouder et al., 2003). This modulatory capacity of sTLR2 may lead to the design of new therapeutics bacteria for the prevention and/or treatment of severe bacteria-induced pathological conditions, including septic shock.

Cloning experiments of mouse TLR4 (mTLR4) have shown that there is an alternatively spliced mTLR4 having an additional exon between the second and third exons with an in-frame stop codon (Iwami et al., 2000). The spliced mRNA encodes 86 amino acids (aa) of the reported mTLR4 and an additional 36 aa. This mRNA encodes a secretary 20-kDa protein known as soluble mTLR4 (smTLR). Treatment with sTLR4 led to significantly reduced LPS-mediated TNF-α production and NF-κB activation. sTLR4 appears to act as a negative bridge between TLR4 and other co-receptors by preventing ligand binding, and may function as a feedback mechanism to inhibit excessive LPS responses in mouse macrophages.

Radioprotective protein 105 (RP105) is a homolog of TLR4 that has a conserved extracellular leucine repeat (Divanovic et al., 2005). RP105 specifically resembles the TLR structure, and was originally discovered as a B cell specific molecule involved in B cell proliferation (Miyake et al., 1994). Unlike TLR4, RP105 lacks a Toll/interleukin-1 receptor (TIR) domain, and instead contains 6-11 intracytoplasmic amino acids. Divanovic et al. (2005) showed that RP105 with this extracellular domain acts as a specific inhibitor of TLR4 signaling in HEK 293 cells, and demonstrated that RP105 forms a complex with MD1 and then interacts directly with the TLR4 complex to inhibit binding of LPS to its receptor. Thus, RP105 adds to the list of promising candidates for the development of new approaches to the treatment of various infectious diseases.

INHIBITORS OF ADAPTOR PROTEINS

Although most members of the IL-1R-TLR superfamily are positive regulators of signaling, the orphan receptor member of this family, ST2, is unable to activate NF-κB. ST2 is able to inhibit NF-κB activation through IL-1RI, TLR2, TLR4 and TLR9, but not TLR3. This specificity of inhibition is achieved by sequestration of the downstream adaptor molecules MyD88 and MAL (Brint et al., 2004). ST2 is expressed in TH2 cells and regulatory T cells, and may be a negative regulator of the adaptive immune response (McGuirk et al., 2002). It has also been shown that ST2 is induced by LPS and that it acts via feedback inhibition. Further studies evaluating the induction and function of ST2 in various cell types should provide important information regarding the therapeutic potential of targeting this molecule during infection and autoimmune diseases. The various proteins that inhibit the TLR adaptor protein are shown in Fig. 2.

Vaccinia virus (VV), the poxvirus used to vaccinate against smallpox, encodes proteins that antagonize important components of the host antiviral defense. VV protein A52R, which has no obvious similarity to host proteins, can block the activation of NF-κB via multiple TLRs, particularly TLR3. A52R associates with both IRAK2 and TRAF6, thereby disrupting signaling complexes containing these proteins. Furthermore, deletion of the *A52R* gene from VV has been shown to reduce virus virulence (Harte et al., 2003). Another protein encoded by VV, A46R, is also capable of inhibiting the binding of various adaptor proteins such as MAL, TRAM, TRIF and MyD88. The structural design of this viral protein might be useful for designing drugs to protect against overactivation of TLRs (Harte et al., 2003; Stack et al., 2005).

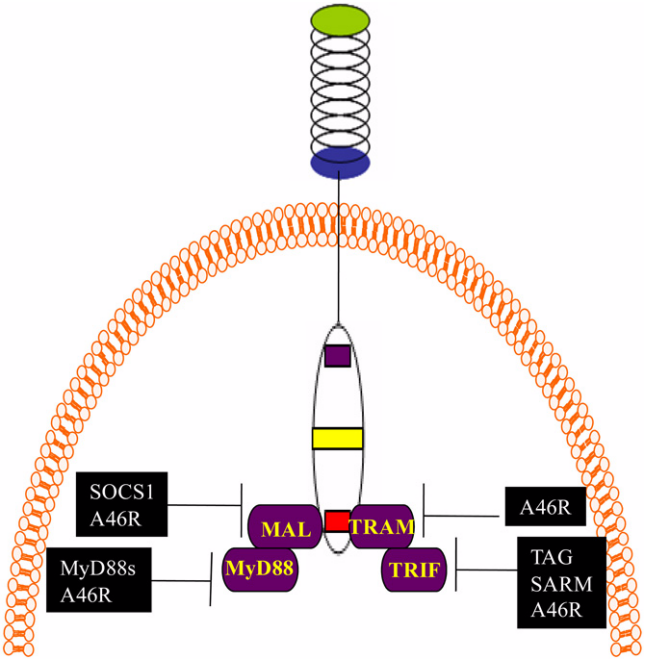

Fig. 2. TLR adaptor proteins. In the simple model of TLR activation, binding of TLR4 ligand LPS induces activation of an inflammatory cascade that leads to the activation and recruitment of adaptor proteins. A46R is a protein secreted by VV that inhibits all four classes of adaptors, including MyD88, MAL TRIF and TRAM. TAG replaces adaptor TRIF, terminating MyD88-independent signaling. The complex of SARM with TRIF prevents it from being involved in TIR interactions with other adaptors or TLRs. MyD88s is a splice variant of MyD88 and lacks an intermediate domain, hence it blocks the interaction of MyD88 with MAL. The SH2 domain of SOCS1 interacts with MAL, thereby inducing its polyubiquitination and subsequent proteasomal degradation.

VVs have evolved a strategy to escape immune surveillance by inhibiting IRAK2 and TRAF6, thereby indirectly inhibiting NF-κB activation (Bowie et al., 2000). VVs have long been known to possess unique strategies for the evasion of host immune responses, including the ability to produce secreted decoy receptors for cytokines such as IL-1, TNF-α, CC chemokines, IFN-α/β and IFN-γ. A52R is more potent than A46R in terms of its capability to interfere with host immune signaling by directly interfering with TIR domain interactions (Bowie et al., 2000). Differences in structure between A52R and A46R may be important in targeting the proteins to different TIR domain-containing signaling molecules.

The product of alternative splicing can act as an inhibitor for its own family member. One well-known example in TLR signaling by alternative splicing is the inhibition of MyD88 by MyD88s, which lacks an intermediate domain and is, therefore, able to act as an inhibitor. MyD88 and MyD88s strongly heterodimerize while still being able to recruit IRAK4, but not to phosphorylate IRAK1 (Janssens et al., 2002). MyD88s is primarily expressed in the spleen and can be induced in monocytes upon LPS treatment. Although MyD88s still binds the IL-1R and IRAK4, it is not able to induce IRAK4 phosphorylation and NF-κB activation. Conversely, MyD88s behaves as a dominant-negative inhibitor of IL-1- and LPS-, but not TNF-α-induced NF-κB activation. Moreover, the regulated expression and antagonistic activity of MyD88s suggests an important role for alternative splicing of MyD88 during regulation of the cellular responses.

Suppressor of cytokine signaling 1 (SOCS1) was originally identified as a negative regulator of the IFN signaling pathway. SOCS1 induces polyubiquitination and degradation of MAL on specific residues through the SH2 domain of SOCS1 (Mansell et al., 2006). TLR ligands directly induce the production of SOCS1, which provides the feedback inhibition of TLR signaling. This complements the delayed production of previously reported inhibitory molecules such as ST2, MyD88s and IRAKM. The rapid shutdown and delayed inhibition of TLR signaling demonstrates that the TLR molecules are subjected to the quantum flux of TLR negative regulators, which confers more protection to the cells from excessive inflammation or clinical manifestations such as autoimmunity and infectious diseases.

Sterile alpha and armadillo motif containing protein (SARM) is an adaptor protein in TLR signaling that acts as an inhibitor of the TRIF-dependent signaling pathway. Knock-out (KO) experiments conducted to evaluate SARM have shown that the activation of TRIF-dependent signaling is followed by secretion of cytokines and chemokines (Carty et al., 2006). LPS treatment enhances the production of SARM within 1 h of stimulation, thus, SARM adds to the family of proteins involved in rapid down-regulation of the TLR-independent pathway. TRIF is a critical adaptor involved in mediation of the TLR3 and TLR4 antiviral responses. For example, the importance of TRIF has been implicated during cytomegalovirus infection (Hoebe et al., 2003a) and up-regulation of the co-stimulatory molecules during LPS treatment (Hoebe et al., 2003b). Moreover, TRIF is targeted by VV protein A46R for immune evasion, as well as by hepatitis C virus protease NS3-4A. Most of the available inhibitors target the MyD88-dependent pathway, but A20 (a deubiquitination enzyme) targets both MyD88 and TRIF signaling followed by SARM in the TRIF-dependent pathway. The serendipitous discovery of SARM adds to the complexity of TLR signaling and results in this protein being the fifth mammalian adaptor identified as a negative regulator of TLR signaling.

Palsson-McDermott et al. (Palsson-McDermott et al., 2009) reported a previously unknown form of TRAM in which a Golgi dynamics (GOLD) domain is spliced amino-terminally into the TIR domain known as the TRAM adaptor with GOLD (TAG) domain. The GOLD domain is a membrane-trafficking domain that localizes proteins to membrane vesicles. TAG displaces TRIF from TRAM, thereby acting as a negative regulator of the MyD88-independent pathway of TLR4 stimulation. TAG is located in the endoplasmic reticulum as well as in the early and late endosomes. The latter study also demonstrated that TRAM dislocates from early endosomes to late endosomes following LPS treatment, where it interacts with TAG. Several splice variants of proteins involved in the TLR signaling pathways have been reported. One such variant of these proteins is MyD88s, which is discussed above. In addition, there exist four different splice variants of IRAK2, two of which (IRAK2c and IRAK2d) (Hardy and O'Neill, 2004) act as inhibitors of TLR signaling upon overexpression. Palsson-McDermott et al. (2009) identified TAG during genomic analysis of TIR domain-containing adaptor molecule 2 (TICAM2) in the human genome. TAG was predicted to lack the first 20 amino acids of TRAM and instead have a GOLD domain at amino acids 41-190, which is believed to mediate protein-protein interactions (Anantharaman and Aravind, 2002). Hence, targeting TAG to boost the immunostimulatory properties of TLR could prove useful, especially in the development of vaccine therapies.

NEGATIVE REGULATION OF TLR SIGNALING MOLECULES

Excessive activation of the TLR signaling pathway contributes to pathogenesis of autoimmune, chronic inflammatory and infectious diseases. TLR signaling and subsequent functions therefore must be under tight negative regulation to maintain immune balance. It has been reported that negative regulation of TLRs can be achieved at multiple levels, and herewith we discuss each of the signaling components which can be targeted for such regulation. Negative regulators of TLR signaling components are depicted as a schematic representation in Fig. 3.

The kinase activity of IRAK1 is inhibited by Toll-like interacting protein (TOLLIP) (Zhang and Ghosh, 2002). TOLLIP is an adaptor protein that inhibits phosphorylation of the IRAK1 protein, thereby negatively regulating TLR signaling. TOLLIP acts in IL-1R signaling by associating with the cytosolic TIR

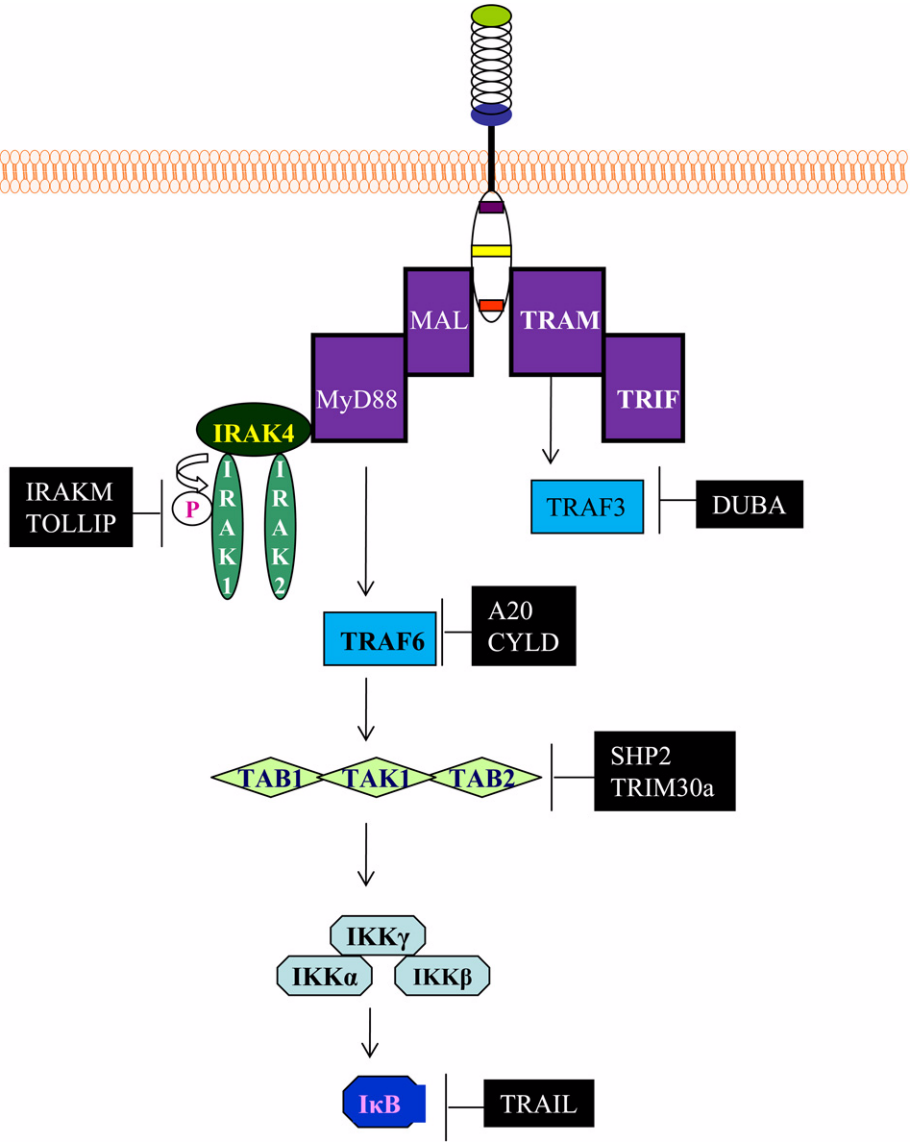

Fig. 3. TLR-induced signaling components. In addition to TLR adaptors, TLR-induced signaling components are under the strict control of physiological negative regulators. IRAKM, a splice variant of IRAK1, acts as an inhibitor of IRAK. The adaptor protein TOLLIP reportedly keeps IRAK1 in an inactive conformation in the absence of TLR/IL-1 receptor stimulation. DUBA selectively cleaves the lysine-63-linked polyubiquitin chains on TRAF3, causing its dissociation from TBK1. A20 protein can remove K63-linked polyubiquitin chains from TRAF6, thereby terminating the NF-κB activation induced by TLR ligands. CYLD negatively regulates TLR mediated immune and inflammatory responses by inhibiting activation of TRAF6 and TRAF7. TRIM30-α degrades TAB2 and TAB3 in an ubiquitin-proteasome pathway independent manner. TRAIL acts as a negative regulator of TLR signaling by stabilizing IκB-α.

domain of IL-1R after IL-1 stimulation (Burns et al., 2000). Upon appropriate stimulation of TLR2 and TLR4, TOLLIP associates directly with those receptors and suppresses the phosphorylation and kinase activity of IRAK1, inhibiting the TLR mediated gene responses. Therefore, TOLLIP may serve as an inhibitor of proinflammatory cytokine production. TOLLIP may mediate the IRAK1 degradation through the presence of an ubiquitin associated (UBA) or coupling of ubiquitin conjugation to endoplasmic reticulum degradation (CUE) domain at the C terminus. This may facilitate the recruitment of ubiquitin dependent enzymes and lead to proteasome dependent degradation in a fashion similar to that of other proteins that undergo ubiquitin dependent degradation (Dieckmann et al., 1998).

A20 is an inducible and broadly expressed cytoplasmic protein that inhibits TNF-α-induced NF-κB activity (Opipari et al., 1990) and was found to play a major role in TNF signaling. However, it was later known for its role in LPS-induced signaling via down-regulation of innate immune responses. A20 performs multiple functions, including the removal of K63 linked polyubiquitin chains from TRAF6 (Boone et al., 2004), facilitating abrogation of the NF-κB responses. A20-deficient mice demonstrate spontaneous inflammation, cachexia and premature death, and A20-deficient fibroblasts cannot properly terminate TNF-α-induced NF-κB activity (Lee et al., 2000). However, the ability of A20 to interact with the signaling molecules TRAF1, TRAF2, TRAF6 and IKK-γ suggests that A20 may also be important for the regulation of other proinflammatory pathways that share these signaling molecules (Heyninck and Beyaert, 1999).

Deubiquitinating enzyme (DUBA) cleaves TRAF3 K63-linked polyubiquitin chains, which prevents its association with TBK1, thereby preventing type I IFN production (Kayagaki et al., 2007). DUBA is an ovarian tumor domain-containing deubiquitinating enzyme that was discovered in a small interfering RNA based screen as a regulator of IFN production. DUBA binds with and selectively cleaves the lysine-63-linked polyubiquitin chains on TRAF3, resulting in its dissociation from the downstream signaling complex containing TBK1. A discrete ubiquitin interaction motif within DUBA is required for efficient deubiquitination of TRAF3 and optimal suppression of type I IFNs.

Cylindramatosis protein (CYLD) is a tumor suppressor that inhibits TRAF6 and TRAF7 induction through TLR2, completely abrogating TLR responses (Yoshida et al., 2005). CYLD also acts as a negative regulator of the *Streptococcus pneumoniae*-induced nuclear factor of activated T-cells (NF-AT) signaling pathway via a deubiquitination-dependent mechanism. CYLD interacts with and deubiquitinates TAK1 to negatively regulate activation of the downstream MKK3/6-p38-α/β pathway.

Tripartite motif-containing protein 30-α (TRIM30-α) belongs to the family of tripartite motif proteins and prevents autodegradation of TRAF6 by targeting TAB1 and TAB2 for degradation (Shi et al., 2008). TRIM30-α promotes the degradation of TAB2 and TAB3, and inhibits NF-κB activation by TLR signaling. Shi et al. (2008) conducted *in vivo* studies of KO mice and further demonstrated that TRIM30-α KO caused impaired LPS induced tolerance. TRIM30-α negatively regulates TLR-mediated NF-κB activation by targeting degradation of TAB2 and TAB3 via a feedback mechanism.

TRAIL (TNF-related apoptosis inducing ligand; or TNFSF10, tumor necrosis factor superfamily, member 10) stabilizes IκB-α, which prolongs NF-κB inactivation and leads to down-regulation of the innate immune response (Diehl et al., 2004). *TRAIL-R*$^{-/-}$ mice develop normal lymphocyte populations, but possess enhanced innate immune responses. *TRAIL-R*$^{-/-}$ mice exhibit increased clearance of the murine cytomegalovirus, which is correlated with increased levels of IL-12, IFN-α, and IFN-γ. Stimulation of macrophages with *Mycobacterium* and activation of TLR2, TLR3 or TLR4, but not TLR9, leads to high levels of TRAIL upregulation and enhanced cytokine production in *TRAIL-R*$^{-/-}$ cells. The immediate-early TLR signaling events in *TRAIL-R*$^{-/-}$ macrophages and dendritic cells are normal, but IκB-α homeostatic regulation and NF-κB activity at later time points are perturbed.

TLR/IL-1 stimulation is associated with increased cytokine production in *IRAKM*$^{-/-}$ cells in response to bacterial infection (Kobayashi et al., 2002). The mechanism of its inhibitory activity includes prevention of the dissociation of IRAK1 from MyD88 after activation. IRAKM expression is restricted to monocytes/macrophages, whereas other IRAKs are ubiquitous. IRAKM prevents dissociation of IRAK and IRAK4 from MyD88 and the formation of IRAK-TRAF6 complexes. *IRAKM*$^{-/-}$ cells exhibit increased cytokine production upon TLR/IL-1 stimulation and bacterial challenge, and *IRAKM*$^{-/-}$ mice show increased inflammatory responses to bacterial infection. Endotoxin tolerance, which is a protective mechanism against endotoxin shock, is significantly reduced in *IRAKM*$^{-/-}$ cells (Kobayashi et al., 2002).

The C-terminal domain of SHP2 binds directly with TBK1 by interacting with its kinase domain. SHP2 deficiency increases TBK1-activated IFN-β and TNF-

α expression, which demonstrates that SHP2 negatively regulates TRIF-mediated gene expression in TLR signaling, partially through inhibiting TBK1 activated signal transduction (An et al., 2006). SHP2 inhibits TLR4/TLR3-activated IFN-β production and TLR3-activated IL-6 and TNF-α production. SHP2 also inhibits poly I:C-induced cytokine production via a phosphatase activity-independent mechanism. Specifically, the C-terminal domain of SHP2 directly binds to TBK1 by interacting with the kinase domain of TBK1. Additionally, SHP2 deficiency increases TBK1-activated IFN-β and TNF-α expression, while TBK1 knockdown inhibits poly I:C-induced IL-6 production in SHP2-deficient cells. SHP2 also inhibits poly I:C-induced activation of the mitogen activated protein kinase pathways. Taken together, these results demonstrate that SHP2 specifically negatively regulates TRIF-mediated gene expression in TLR signaling through the inhibition of the TBK1-activated signal transduction.

INHIBITORS OF TRANSCRIPTION FACTORS

As discussed in the above sections, TLR signaling molecules can be subjected to inhibition at each and every level of their activation. Some of the better established ones for the inhibition of transcription factors are described below and overall summery is shown in Fig. 4.

Peptidylprolyl cis/trans isomerase, NIMA-interacting 1 (PIN1), negatively regulates IRF3 activation via phosphorylation of the Ser339-Pro340 motif, leading to polyubiquitination and proteasome dependent degradation of IRF3 when stimulated with dsRNA (Saitoh et al., 2006). After stimulation with dsRNA, phosphorylation of the Ser339-Pro340 motif of IRF3 leads to its interaction with PIN1 and, finally, poly-

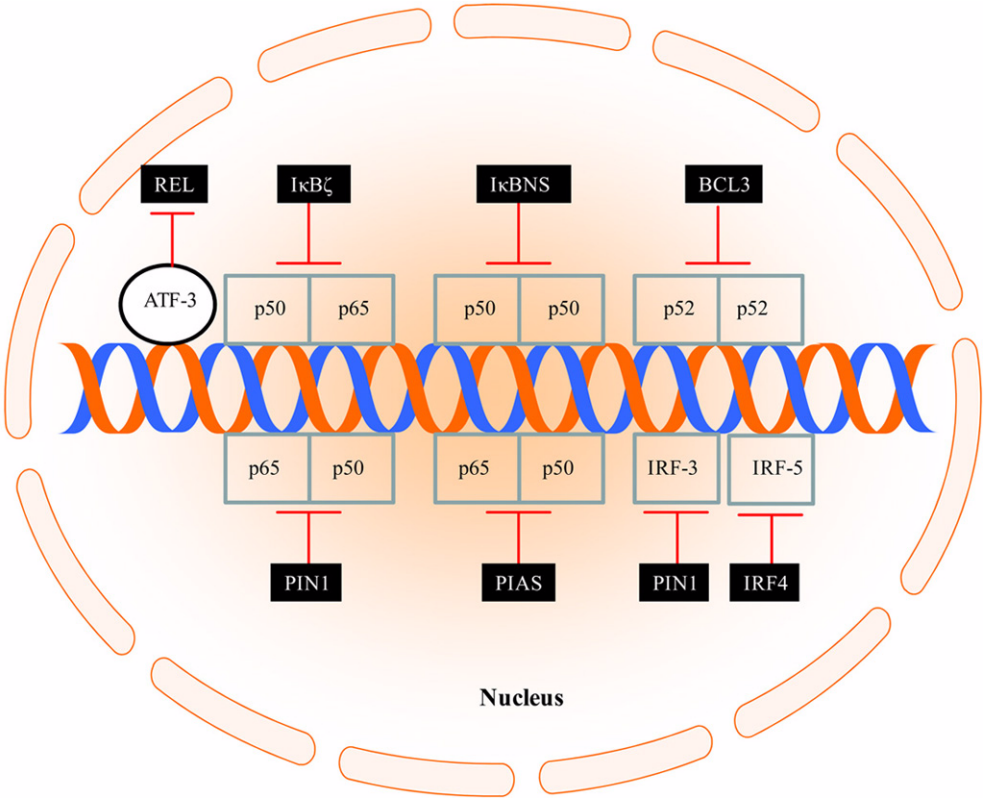

Fig. 4. Inhibitors of transcription factors. IκBNS suppresses the production of IL-6 by binding with DNA bound p50/p50 homodimers. IκB-ζ is unable to transactivate p65 and acts as a negative regulator. ATF3, which is produced during TLR4 stimulation, binds with promoters of IL-6 and IL-12 to recruit HDAC, thereby closing chromatin for the accession of transcription factors such as REL. BCL3 binds with p50/p50 and p52/p52 homodimers to stabilize and prolong their occupancy on the promoters, thereby inhibiting NF-κB dependent transcription. PIN1 prevents ECS-induced ubiquitination and degradation of nuclear p65 containing complexes. PIN1 also leads to the polyubiquitination and proteasome dependent degradation of IRF3. The PIAS family of proteins including PIAS1 and PIASy (PIAS4) has been shown to affect NF-κB activity. IRF5 and IRF7 interact with MyD88 and produce proinflammatory cytokines and type I IFNs. In this case, IRF4 competes with IRF5, but not with IRF7, for MyD88 interaction, thereby acting as a negative regulator of TLR signaling.

ubiquitination and subsequent proteasome-dependent degradation of IRF3. Suppression of PIN1 by RNA interference or genetic deletion results in enhanced IRF3-dependent production of IFN-β, with a consequent reduction in virus replication. These results elucidate a previously unknown mechanism for controlling innate antiviral responses by negatively regulating IRF3 activity via PIN1.

IRF4 also interacts with MyD88 and acts as a negative regulator of TLR signaling. IRF4 mRNA is induced by TLR activation, and IRF4 competes with IRF5, but not with IRF7, for MyD88 interaction (Negishi et al., 2005). The TLR-dependent induction of proinflammatory cytokines is markedly enhanced in peritoneal macrophages from mice deficient in the *IRF4* gene, whereas the induction is inhibited by the ectopic expression of IRF4 in a macrophage cell line. The critical function of IRF4 in TLR signaling *in vivo* is underscored by the observation that IRF4 deficient mice show hypersensitivity to DNA-induced shock with elevated serum proinflammatory cytokine levels. This may provide an insight into the complex regulatory mechanisms of MyD88 signaling by IRFs.

BCL3 (B cell leukemia/lymphoma 3) was identified as a proto-oncogene that also functions as an inhibitor of NF-κB activity by stabilizing repressive homodimers of NF-κB in DNA promoters. BCL3 prevents the ubiquitination of p50 homodimers where it exhibits strong binding tendency. Specifically, macrophages treated with IL-10 produce BCL3, which inhibits LPS-induced TNF-α production (Kuwata et al., 2003). BCL3 also inhibits LPS-induced production of TNF-α, but not IL-6, in macrophages. In BCL3 transduced and IL-10 pretreated macrophages, LPS-induced nuclear translocation of NF-κB p65 was not found to be impaired, but DNA binding by NF-κB p50/p65 was profoundly inhibited. BCL3 interacts with NF-κB p50 and is recruited to the TNF-α promoter, but not the IL-6 promoter, indicating that BCL3 facilitates p50-mediated inhibition of TNF-α expression. These findings suggest that IL-10-induced BCL3 is required for the suppression of TNF-α production in macrophages.

Another interesting aspect of the control of inflammation is the discovery of activating transcription factor 3 (ATF3). ATF3 belongs to the ATF3/CREB family of transcription factors and inhibits LPS-induced IL-6 and IL-12 production, acting as a negative regulator of TLR4 signaling (Gilchrist et al., 2006). *In vivo* experiments have shown that ATF3 protects mice from LPS-induced endotoxic shock. ATF3 appears to function by recruiting histone deacetylases (HDACs) to the gene promoters, thereby limiting the accession of transcription factors (Gilchrist et al., 2006). Acetylation opens the chromatin for the accession of transcription factors, while deacetylation closes the chromatin. In addition to acting as a negative regulator of TLR4 signaling, ATF3 has also been found to act as a negative regulator of the TLR2/6, TLR3, TLR5, TLR7 and TLR9 signaling pathways (Whitmore et al., 2007).

Nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor, delta (NF-κBID or IκBNS) suppresses IL-6 production by binding with DNA bound p50 homodimers, thereby preventing the binding of functional NF-κB dimmers in the DNA (Hirotani et al., 2005). Lentiviral introduction of IκBNS results in impaired LPS-induced IL-6 production, but not TNF-α production in the RAW264.7 murine macrophage cell line. IκBNS expression leads to constitutive and intense DNA binding of NF-κB p50/p50 homodimers. IκBNS is recruited to the IL-6 promoter, but not the TNF-α promoter, together with p50. Furthermore, small interfering RNA-mediated reduction in IκBNS expression in RAW264.7 cells results in increased LPS-induced production of IL-6, but not TNF-α. Thus, IκBNS selectively suppresses LPS-induced IL-6 production in macrophages.

Nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor, zeta (NF-κBIζ or IκB-ζ) negatively regulates gene expression when bound with p65 containing complexes because it prevents transactivation (Motoyama et al., 2005). Reporter analysis revealed that IκB-ζ inhibits the activation of NF-κB. Further molecular experiments indicated that the carboxy-terminal domain with ankyrin repeats inhibits the DNA binding of p65/p50 heterodimer and p50/p50 homodimer (Yamazaki et al., 2001).

The protein inhibitor of activated STAT (PIAS) family consists of four members, including PIAS1, PIAS3, PIASx and PIASy (Chung et al., 1997; Liu et al., 1998). Initially, the roles of the PIAS family proteins were identified due to their participation in inhibition of the STAT proteins, and subsequent studies have suggested that the PIAS proteins play a role in the regulation of a variety of transcription factors (Sharrocks, 2006). The specific roles of PIAS proteins were identified based on their negative regulation of NF-κB/STAT1 signaling during innate immune responses (Sharrocks, 2006). PIAS1 acts by selectively inhibiting the recruitment of NF-κB/STAT1 to the endogenous gene promoters (Liu et al., 2004). Despite the well-documented role of PIAS1, its contribution to innate immunity remains largely unknown. PIAS belongs to the small ubiquitin like modifier (SUMO) family of ligases, and this ligase activity is important for the phosphorylation of PIAS

and subsequent repression of NF-κB and STAT1 dependent transcriptional responses (Liu et al., 2007).

PIN1 prevents elongin-B-elongin-C-cullin-2-SOCS1 (ECS)-induced ubiquitination and ubiquitin-mediated proteolysis of p65 containing NF-κB dimers. Following cytokine treatment, PIN1 specifically binds to the pThr254-Pro motif in p65, inhibits p65 binding to IκB-α, and enhances p65 nuclear localization and protein stability (Ryo et al., 2003b). Significantly, deregulation of PIN1-catalyzed prolyl isomerization and ubiquitin-mediated proteolysis of p65 may offer new insights into abnormal activation of NF-κB in human diseases, particularly cancer, which is known to cause increased expression of PIN1 (Ryo et al., 2001; Ryo et al., 2002; Ryo et al., 2003a). PIN1-deficient mice and cells are refractory to NF-κB activation by cytokine signals. Moreover, the stability of p65 is controlled by ubiquitin-mediated proteolysis and facilitated by a cytokine signal inhibitor, SOCS1, which acts as ubiquitin ligase (Ryo et al., 2003b) and is silenced in many human malignancies (Rottapel et al., 2002; Yoshikawa et al., 2001).

TLRs AND CANCER

TLRs are not only activated in normal cells, but also in tumor cells that have been stimulated with its cognate ligand. Table II highlights the molecules involved in TLR signaling in cancer cells. TLRs expressed in cancer cells have both positive and negative effects (Fig. 5). TLRs induce increased resistance to apoptosis and increased tendency towards invasiveness in cancer cells. In ovarian cancer cells, an increased expression of X-linked inhibitor of apoptosis protein (XIAP), a major caspase 3 and 9 inhibitor, and a high level of phosphorylated AKT occur during TLR4 signaling (Yuan et al., 2003; Dan et al., 2004). This mechanism renders the ovarian cancer cells more resistant to tumor growth and causes the development of chemoresistance (Dan et al., 2004). Matrix metalloproteinase 13 (MMP13) is a major contributor to MDA-MB-231 breast cancer cell metastasis and prostate cancer cell invasion through activation by TLR9 (Ilvesaro et al., 2007; Merrell et al., 2006). Additionally, the results of several studies suggest that TLR4 and TLR9 play a pivotal role in spreading cancer via the upregulation of inducible nitric oxide synthase (iNOS), MMP2 and beta1 integrin subunits when stimulated with LPS (Harmey et al., 2002; Wang et al., 2003). In addition, the endogenous/exogenous ligands reported for TLRs include HSP60 (Kol et al., 2000), HSP70 (Asea et al., 2000), HSP90 (Singh-Jasuja et al., 2000), MHC class I and II molecules (Singh-Jasuja et al., 2000; Asea et al., 2000; Kol et al., 2000), CD80 and CD86 (Singh-Jasuja et al., 2000), fibrinogen (Smiley et al., 2001; Guillot et al., 2002), surfactant protein A (Guillot et al., 2002), domain A of fibronectin (Okamura et al., 2001), heparan sulfate (Johnson et al., 2002; Okamura et al., 2001), beta-defensin 2 (Biragyn et al., 2002), high mobility group box 1 pro-

Table II. TLR signaling molecules implicated in cancer

TLR signaling molecules	Cancer	TLRs	References
Beta-defensin 2	Oral cancer	TLR2/TLR4	Biragyn et al., 2002
CD80 and CD86	All cancer	TLR2/TLR4	Asea et al., 2000; Kol et al., 2000; Singh-jasuja et al., 2000
Domain A of fibronectin	All cancer	TLR2/TLR4	Okamura et al., 2001
Fibrinogen	All cancer	TLR2/TLR4	Smiley et al., 2001
Heparan sulfate	All cancer	TLR2/TLR4	Johnson et al., 2002
High mobility group box 1 protein	All cancer	TLR2/TLR4	Park et al., 2004
HSP60	All cancer	TLR2/TLR4	Asea et al., 2000; Kol et al., 2000; Singh-jasuja et al., 2000
HSP70	All cancer	TLR2/TLR4	Asea et al., 2000; Kol et al., 2000; Singh-jasuja et al., 2000
HSP90	All cancer	TLR2/TLR4	Asea et al., 2000; Kol et al., 2000; Singh-jasuja et al., 2000
MHC class I and II molecules	All cancer	TLR2/TLR4	Asea et al., 2000; Kol et al., 2000; Singh-jasuja et al., 2000
Surfactant protein A	Lung cancer	TLR2/TLR4	Guillot et al., 2002
AKT	Ovarian cancer cell	TLR4	Yuan et al., 2003; Dan et al., 2004
Beta1 integrin subunit	All cancer	TLR4	Harmey et al., 2002; Wang et al., 2003
iNOS	All cancer	TLR4	Harmey et al., 2002; Wang et al., 2003
MMP2	All cancer	TLR4	Harmey et al., 2002; Wang et al., 2003
X-linked inhibitor of apoptosis	Ovarian cancer cell	TLR4	Yuan et al., 2003; Dan et al., 2004
Indolamine 2,3-dioxygenase	Cancer	TLR9	Mellor et al., 2005; Wingender et al., 2006
MMP13	Breast cancer cell	TLR9	Merrel et al., 2006
MMP13	Prostate cancer	TLR9	Ilversaro et al., 2007

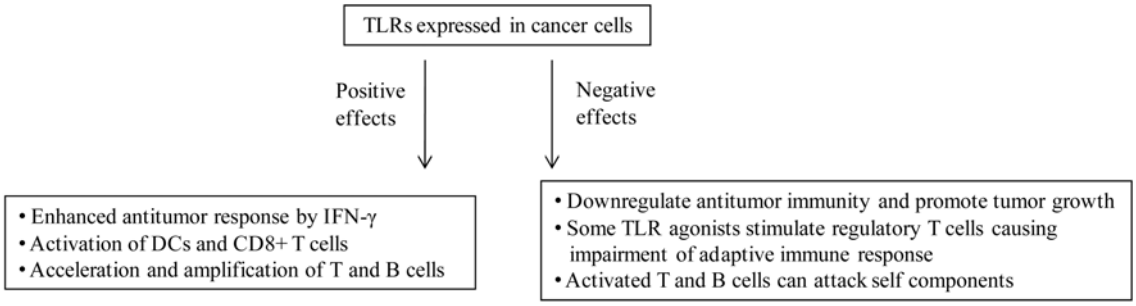

Fig. 5. Positive and negative effects of TLRs in cancer cells. TLRs enhance antiviral responses, and simultaneously enhance the tumor growth and invasion. For example, the MDA-MB-231 breast cancer cell line expresses TLR9, which leads to the secretion of MMP13 when activated, thus causing cell invasion. Similarly, tumor cells express TLR4 which produces IL-6, NO and IL-12 when stimulated. This, in turn, enables cells to mimic the inflammatory cell response and escape immune cell surveillance.

tein (Park et al., 2004), and indolamine 2,3-dioxygenase (Mellor et al., 2005; Wingender et al., 2006). These findings demonstrate the widespread effects of TLRs and urge the development of TLR-based drugs for cancer therapy.

TLR AGONISTS AS ADJUVANTS IN VACCINE DEVELOPMENT

The major concern with vaccine therapy is once they are injected in its "pure" form (which contain only target antigen to reduce the side-effects of vaccine such as fever, swelling and pain), they are less effective in eliciting immunogenicity. Therefore, it is very much necessary to add some immunological agents to be used with vaccines to stimulate the innate immune system and such agents are called as "adjuvants" (Fig. 6). Adjuvants when used with vaccines have shown to be very powerful in enhancing immune responses, thus helping to lower the concentration of the vaccine (Hunter, 2002; Rappuoli, 2007). Once injected, these adjuvants use immune receptors expressed on antigen presenting cells (APCs) such as dendritic cells and macrophages (Ishii and Akira, 2007). Synthetic nucleic acids (poly I:C and CpG-ODN) are known to be adjuvants that induce proinflammatory cytokines and interferon responses. In the following section we will discuss the current adjuvants and their status.

Many TLR ligands have been used as adjuvants in various vaccines to promote immune responses. Some of these have been shown to be effective in cancer immunotherapy. However, when such agonists are used, it is possible to trigger non-TLR cytosolic pattern recognition receptors. Although LPS has been produced as an adjuvant, it is difficult to clinically test such adjuvants because LPS is highly toxic to humans and has limited options for use with vaccines (Masihi et al., 1986; Cluff et al., 2005). Nevertheless, efforts have been directed towards the development of purified components from LPS by eliminating lipid A, a highly active toxic component in the LPS structure. This

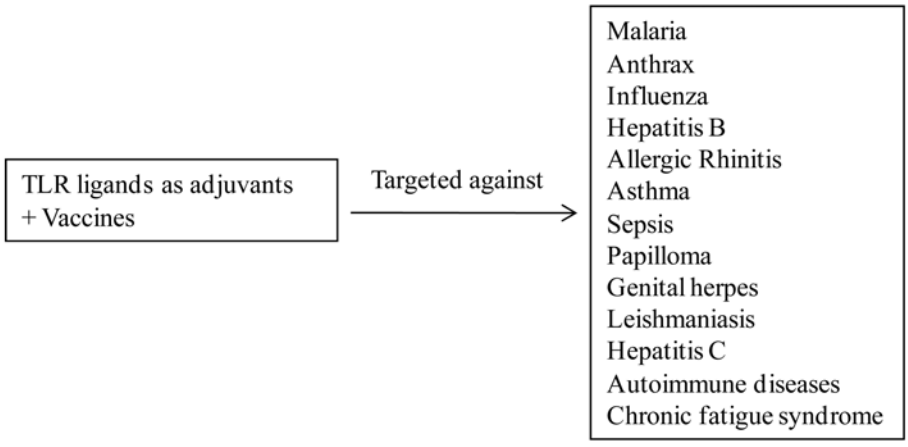

Fig. 6. TLR ligands as adjuvants. Various TLR ligands are currently being used as adjuvants in vaccine therapies, where they have shown promising effects without causing overactivation of immune system.

approach has led to the development of monophosphoryl lipid A (MPL)-based adjuvant (monophosphoryl lipid A/trehalose dicorynomycolate; 'Ribi' adjuvant), which is a clinically tested adjuvant used with human vaccines effective against infectious diseases and rhinitis (Evans et al., 2003; Hoffman et al., 2005). OK-432 from *Streptococcus* activates TLR4 and enhances the antitumor effects of IFN-γ (Okamoto et al., 2006). *Hemophilus influenzae* type B (HIP-OMPC) and outer surface lipoprotein of *Borrelia burgdorferi*, which are used as vaccines, stimulate TLR2 (Yoder et al., 2003; Latz et al., 2004). Cytoplasmic pattern recognition receptors play a pivotal role in the recognition of adjuvants as well. For example, muramyl dipeptide (MDP), a component of bacterial peptidoglycan (PGN), and desmuralmylpeptides containing diaminopimelic acid (DAP), another structural component of PGN, are recognized by NOD1 and NOD2, respectively (Inohara et al., 2005; Fritz et al., 2006). Flagellin, which is found on the outer structure of bacteria, is recognized by TLR5, and it has been shown to be an effective adjuvant due to its protein nature. Additionally, some DNA vaccines containing a chimeric version of both antigenic protein and flagellin have been developed (Applequist et al., 2005; Honko et al., 2006). Poly I:C has been evaluated in clinical trials to treat human immunodeficiency virus (HIV) and leukemia, but its high toxicity has prevented further use (Robinson et al., 1976). Nevertheless, studies are still being conducted to lower the toxicity of poly I:C against breast and ovarian cancer (Adams et al., 2003). The ligand of TLR9 has been actively investigated for its use as an adjuvant in vaccines. CpG-ODN elicits strong immune responses by activating CD4 Th1 and CD8 CTL, making it useful for vaccine adjuvants and anti-allergens. Indeed, when co-administered with allergens and vaccines, CpG-ODN has been effective in preclinical studies (Broide, 2005; Vollmer, 2006). ITMN-191 is a peptidomimetic inhibitor of the NS3/4A protease of hepatitis C virus (HCV), which is currently under preclinical studies developed by Array Biopharma (Colorado, USA) (Seiwert et al., 2008).

TLR DRUGS UNDER DEVELOPMENT

There are currently active investigations being conducted to evaluate the use of TLR ligands against infectious disorders, allergies, cancers and autoimmune diseases (Table III). TLR-based vaccines for malaria, anthrax, influenza and hepatitis B virus (HBV) are being evaluated in clinical trials (Kanzler et al., 2007; Krieg, 2006; Higgins et al., 2007; Meyer and Stockfleth, 2008). HBV vaccine and CpG-B ISS (CpG-7909) against HBV and HIV infection are being evaluated in phase III and phase I/II clinical trials, respectively. CYT005-AllQbG10 is a vaccine combined with a CpG ligand that has led to the robust induction of Th1 responses. Indeed, treatment of nine patients showed positive effects against allergic rhinitis and asthma. VaxImmune has completed phase I studies and is entering into phase III clinical trials. VaxImmune enhances antibody induced immune responses and elevates the potency of T cell killer responses. Heplisav is in clinical phase III trials to evaluate the effectiveness of its responses against HBV (Halperin et al., 2006). Furthermore, TLR9 agonists have already shown to be useful as a malaria vaccine (Mullen et al., 2006). TLR4 is the only receptor known to have antagonists that have been tested clinically. Eritoran (Eisai) and Resartorvid are two TLR4 inhibitor compounds that have shown promising tolerance to sepsis and which have entered phase I clinical trials (Ondiveeran and Fox-Robichaud, 2004; Kaplan and Tipirneni, 2007). The use of vaccines and agonists of TLR ligands has been well-studied in mice, but has been evaluated in only a few human clinical trials. The ability of the TLR7 agonist to induce a large amount of interferons provides cells with the opportunity to fight against infections. Imiqumoid is a TLR7 agonist that has already been approved for its actions in preventing papilloma-induced genital warts (Chang et al., 2005), molluscum contagiosum, genital herpes and leishmaniasis (Meyer and Stockfleth, 2008). ANA975 (Isatoribine) is an agonist of TLR7 that activates innate immunity and has proven to be effective in HCV infection (Xiang et al., 2007). In a clinical study of chronic HCV infection, this drug caused a decrease in the HCV viral RNA concentration in plasma. Moreover, intravenous administration of this drug further decreased the viral load in a fashion similar to IFN-α based treatment. ANA773 is a TLR7 agonist prodrug that is about to begin phase I clinical trials in The Netherlands to evaluate its effectiveness against HCV infection. Innate Pharma is actively engaged in the development of a TLR7 agonist IPH-32XX to fight cancers, autoimmune diseases and infectious diseases. Ampligen (poly I: poly C12U) is a TLR3 agonist that has been tested against chronic fatigue syndrome. Finally, CQ-07001 is a powerful TLR3 agonist that is currently undergoing preclinical development.

CONCLUSION

TLRs have been targeted for the treatment of many diseases. Indeed, some naturally occurring negative and positive regulators of TLR signaling have been

Table III. TLR drugs

TLR Target	Drug Company or Institute	Drug Name	Disease	Status
TLR3	Clinquest (http://www.clinquest.com)	CQ-07001		Preclinical
TLR3	Hemispherx Biopharma (http://www.hemispherx.net)	Ampligen poly(I):poly(C12U)	Chronic fatigue syndrome	Phase II
TLR3	Innate Pharma (http://www.innate-pharma.com)	IPH-31XX	Cancer	Preclinical
TLR3	Multicell Technologies (http://www.multicelltech.com)	MCT-465	Viral or oncology vaccines	Preclinical
TLR4	Eisai (http://www.eisai.com)	Eritoran	Sepsis	Phase III
TLR4	Takeda (http://www.takeda.com)	TAK-242	Sepsis	Phase III
TLR5	VaxInnate (http://www.vaxinnate.com)	Flagellin HuHa	Influenza	Preclinical
TLR5	VaxInnate (http://www.vaxinnate.com)	Flagellin HuM2e	Influenza A	Preclinical
TLR7	3M Pharmaceuticals/Graceway Pharmaceuticals (http://www.gracewaypharma.com)	Aldara™	Basal cell carcinoma	Approved
TLR7	Anadys (http://www.anadyspharma.com)	ANA773	Cancer	Preclinical development
TLR7	Anadys (http://www.anadyspharma.com)	ANA975 (Isatoribine)	HCV	Phase Ib
TLR7	Innate Pharma (http://www.innate-pharma.com)	IPH-32XX		
TLR9	Anadys (http://www.anadyspharma.com)	ANA733	Hepatitis C	Phase I
TLR9	Coley Pharmaceutical (http://www.coleypharma.com)/Pfizer (http://www.pfizer.com)	CpG-10101	Hepatitis C	Phase I suspended
TLR9	Coley Pharmaceutical (http://www.coleypharma.com) /Pfizer (http://www.pfizer.com)	PF-3512676	Non-small cell carcinoma	Phase III
TLR9	Coley Pharmaceutical (http://www.coleypharma.com)/Pfizer (http://www.pfizer.com)	VaxImmune™	Anthrax	Phase I
TLR9	Cytos Biotechnology (http://www.cytos.com)	CYT004-MelQbG10	Melanoma	Phase IIa
TLR9	Cytos Biotechnology (http://www.cytos.com)	CYT005-AllQbG10	Allergic rhinoconjunctivitis, Asthma	
TLR9	Dynavax Techologies (http://www.dynavax.com)	1018ISS	Nonhodgkin's lymphoma	Phase II completed
TLR9	Dynavax Techologies (http://www.dynavax.com)	1018ISS	Metastatic colorectal cancer	Phase I completed
TLR9	Dynavax Techologies (http://www.dynavax.com)	CpG-B and CpG-C class ODN	Hepatitis C	Preclinical
TLR9	Dynavax Techologies (http://www.dynavax.com)	Heplisav™	Hepatitis B	Phase III
TLR9	Dynavax Techologies (http://www.dynavax.com)	Influenza antigents and CpG-ISS	Influenza	Preclinical
TLR9	Dynavax Techologies (http://www.dynavax.com)	Second generation CpG-ISS	Asthma	Preclinical
TLR9	Dynavax Techologies (http://www.dynavax.com)	Tolamba	Allergic rhinitis (ragweed)	Phase II/III completed
TLR9	GlaxoSmithKline (http://www.gsk.com)	MAGE-3AplusCpG-B	Non-small cell lung cancer	Phase III
TLR9	Idera Pharmaceuticals (http://www.iderapharma.com)	Amplivax	HIV	Phase II
TLR9	Idera Pharmaceuticals (http://www.iderapharma.com)	IMO-2125	Hepatitis C	Phase I
TLR9	Idera Pharmaceuticals (http://www.iderapharma.com)	IMOxine®	Non-small cell lung cancer	Phase I
TLR9	Idera Pharmaceuticals (http://www.iderapharma.com)	IMO-2055	Renal cell carcinoma	Phase II
TLR9	Idera Pharmaceuticals (http://www.iderapharma.com)/ Novartis (http://www.novartis.com)	HYB2093	Asthma	Preclinical
TLR9	NIAID (http://www3.niaid.nih.gov)	AMA1-C1/Alhydrogel®	Malaria	Phase completed
TLR9	Sanofi-Aventis (http://en.sanofi-aventis.com)	AVE0675, AVE7279	Atopic diseases	

shown to be effective agents for regulation of innate immunity. Researchs into the regulators of innate immunity may shed more light on the discovery of a natural green pharmacy (Green Pharm). Such a Green Pharm could be a very effective means of therapy for various diseases that also helps communities safely reduce the environmental impact of pharmaceutical waste.

ACKNOWLEDGEMENTS

This work was supported by the Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the the Ministry of Education, Science and Technology (2009-0075674). This work was also partly supported by the Korea Science and Engineering Foundation (KOSEF) (R01-2007-000-20533-0), the Priority Research Centers Program through the NRF (2009-0093826), and the Ajou University Research Fellowship.

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