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Toll Like Receptors in Dual Role: Good Cop and Bad Cop

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1. Introduction

Every living organism tends to protect itself from harmful effects of pathogens or molecules of pathogenic origin that can disturb its well-being state. The first line of defence that comes into action upon encounter with the pathogen is referred to as innate immune defence mechanism. It had been a matter of great inquisitiveness how innate immune defence mechanism is able to render the body protected against such a diverse variety of pathogens. But with the discovery of germ line encoded pattern recognition receptors (PRRs) that can sense the pathogen associated molecular patterns (PAMPs), it is to an extent possible to answer the query, how innate immune system copes to recognise such a wide variety of micro-organisms and harmful microbial elements. PAMPs are usually of pathogenic origin and absent from the cells of host origin. PRRs can be transmembrane receptors like Toll like receptors (TLRs) (Beutler & Rietschel, 2003; Janeway & Medzhitov, 2002), C-type lectin receptors (CLRs) or these can be cytosolic receptors like Nod like receptors (NLRs) and Rig like helicases (RLRs). Every PRR is capable of recognising specific conserved molecular patterns on the micro-organism and later can start a downstream signalling process upon proper interaction of PAMP and PRR that leads to synthesis of effector molecules like antimicrobial peptides and pro-inflammatory cytokines that prevent the body from otherwise harmful microbes.

In the late 90's a protein was discovered in *Drosophila* named as Toll. Toll is a transmembrane receptor that is required for the establishment of proper dorso-ventral polarity during embryo formation in *Drosophila* (Hashimoto et al., 1988). Mutation in Toll gene results in a weird phenotype of the fruitflies. Later it was found that signalling pathways of *Drosophila* Toll and mammalian IL-1 receptor showed marked resemblance leading to the assumption that Toll may be involved in the regulation of immune responses. Now, it is well established that Toll signalling is required for the defence against Gram-positive, Gram-negative bacterial and fungal infections. Toll is responsible for the production of Drosomycin, antifungal peptide (Lemaitre et al., 1996). Mutants lacking in components of Toll mediated signalling pathway (Toll, Spatzle, Tube, Pelle) are highly susceptible to fungal infections. A year succeeding the discovery of Toll in *Drosophila*, through database searches, Toll homologues in mammals as well were revealed known as

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Toll like receptors (TLRs). TLRs recognise PAMPs of diverse origin from bacteria, virus, fungi, protozoa and others. TLRs can also sense the molecules that are generated within the host cells alarming a sort of danger signal like heat shock proteins (Hsp60, Hsp70, Hsp90), fibrinogen, surfactant protein A, heparin sulphate and others. Thirteen TLRs are reported so far, out of which TLR1 to TLR9 are conserved between human and mice. TLR10 is only functional in human while TLR11 is found to be functional only in mice. Upon interaction with their cognate ligands, TLRs either homodimerise or heterodimerise to further proceed the downstream signalling.

2. Structure of TLRs

Toll like receptors are type-I transmembrane receptors having an extracellular domain containing multiple leucine rich repeats (LRRs). There are about 19-25 tandem repeats of LRR motif each having 20-29 residue sequence motif LXXLXLXXNXLXXLXXXXXXXLXX where X is any amino acid (Bell et al., 2003). LRR motifs are responsible for interacting and recognising specific ligands and thereby initiating downstream signal transduction. LRRs are varied among different TLRs enabling them to sense a wide variety of PAMPs. Interaction of the pathogen with the LRR motif is supposed to take place at the concave side of the horse shoe shaped LRR motif. Mammalian TLRs are found to have homology with IL-1 receptor in cytoplasmic domain known as Toll/IL-1R or TIR domain while extracellular regions are devoid of any homology having three immunoglobulin domains in IL-1R and LRR motifs in TLRs. TIR domain consists of about 200 amino acids (Slack et al., 2000) and is composed of five β strands (βA, βB, βC, βD and βE) alternated with five α helices (αA, αB, αC, αD and αE) (Xu et al., 2000) connected via 8 loops. Box1, Box2 and Box3 are three highly conserved regions found to be present in TIR domain. BB loop is formed when Box2 forms a loop connecting the second β strand and α helix. This BB loop is of primary importance in further downstream signalling because any single amino acid residue substitution in this loop can lead to the complete impairment of its function. In C3H/HeJ mice, a point mutation in BB loop replacing conserved proline leads to hypo-responsiveness to the LPS resulting in loss of function of BB loop (Poltorak et al., 1998).

3. Distribution of TLRs

TLRs are generally expressed on the cells of innate immune system like dendritic cells, monocytes and macrophages (Beutler & Rehli, 2002) that are likely to have interacted with the pathogen earlier. TLR expression is found to be highest on the phagocytic cells like tissue macrophages, neutrophils and dendritic cells. Macrophages express all TLRs except TLR3. However, not all TLRs are expressed by all cell types i.e. TLR expression is tissue specific eg. TLR5 is shown to be exclusively expressed on the intestinal epithelial cells' basolateral surface. Also TLR expression may vary with the maturation stage of the cell, eg. TLR1, 2, 4 and 5 are shown to be expressed on the immature dendritic cells but there expression decreases as the cells undergo maturation. TLR3 is shown to be expressed only on mature dendritic cells. Yet tissue specific demarcation of TLR expression is not clear, it is observed that most of the tissues express atleast one type of TLR. Also TLR expression is found to be different in the two subsets of blood dendritic cells i.e. Myeloid dendritic cells express TLR1, 2, 4, 5 and 8 while plasmacytoid dendritic cell express TLR7 and TLR9 exclusively. TLR2 and 4 are highly expressed on the surface of macrophages but are also reported to be expressed on the endothelial cells, smooth muscle cells, intestinal cells and

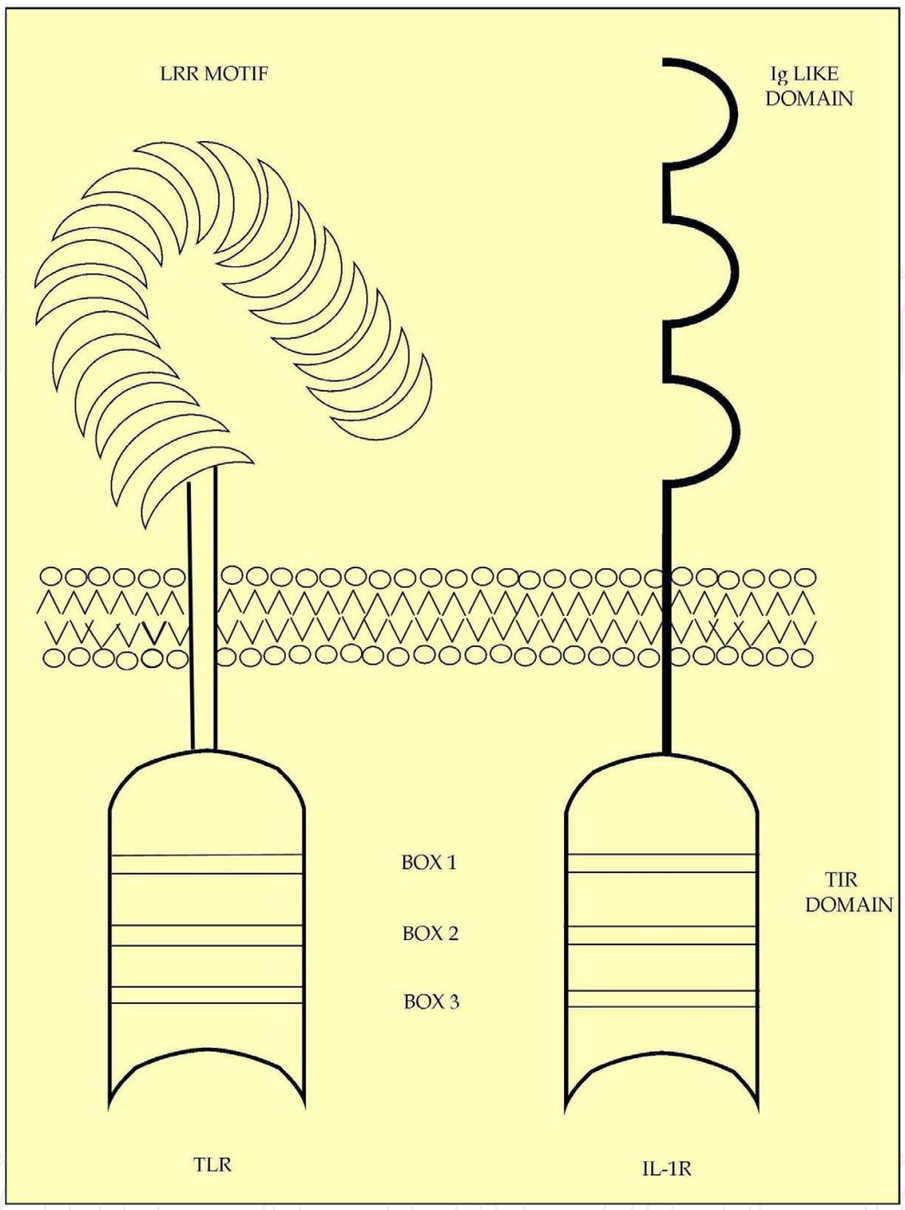


Fig. 1. Toll like receptors and IL-1R are transmembrane receptors both having a conserved region of about 200 amino acids in their cytoplasmic domain known as TIR domain. Three highly conserved regions in TIR domain are referred to as Box1, Box2 and Box3. TLRs and IL-1R though similar in their cytoplasmic domains are markedly different in their extracellular components; TLRs have LRR motif and IL-1R has Ig like domain extracellularly.

others. Studies also indicate subcellular location of TLRs. TLR1, TLR2, TLR4, TLR5 and TLR6 have been found to be expressed on the cell surface, as demonstrated by positive staining of the cell surface by specific antibodies and these recognize bacterial products while TLR3, TLR7, TLR8 and TLR9 have been shown to be expressed in intracellular compartments such as endosomes and recognize microbial nucleic acids (Takeda & Akira, 2005).

4. Phylogenetic relationship among TLRs

A sequence similarity search of different human TLRs revealed that TLRs can be subdivided into five subfamilies i.e. TLR2, TLR3, TLR4, TLR5 and TLR9 subfamilies. While the TLR3, TLR4 and TLR5 are the only respective members of their subfamily, TLR2 subfamily comprises of four members viz. TLR1, TLR2, TLR6 and TLR10; TLR9 family has three members TLR7, TLR8 and TLR9. Members within a subfamily exhibit high ratio of similar sequences than members of other subfamily eg. TLR1 and TLR6 show about 70% similarity in their amino acid sequence, identity approaches about 90% in their TIR domains.

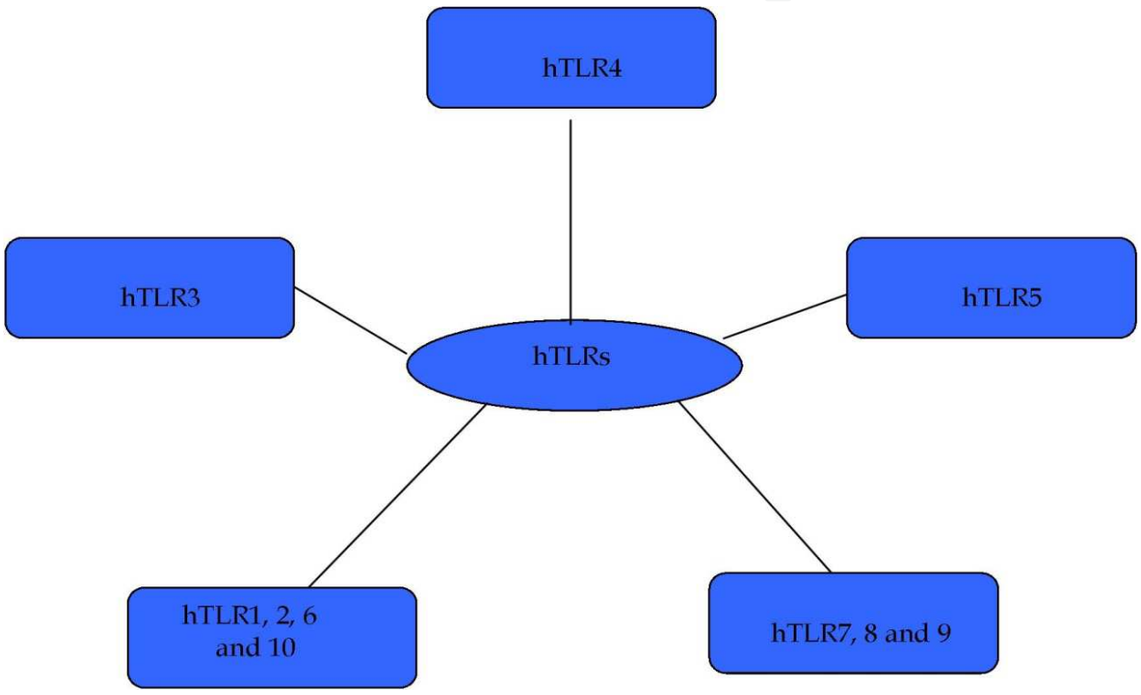


Fig. 2. Human TLRs can be divided into five subfamilies- TLR2, TLR3, TLR4, TLR5 and TLR9. Division is based on the amino acid sequence similarity.

5. Ligands of TLRs

TLRs are able to recognise a wide variety of pathogens and thereafter signal transduction commences that leads to mounting of desirable immune response against the pathogen like expression of inflammatory cytokines, chemokines, antibacterial peptides, enhanced expression of co-stimulatory molecules etc. Generally every TLR recognises more than one type of PAMP e.g. TLR4 (first TLR to be discovered in mammals) has the ability to recognise a variety of PAMPs diverse in nature, for instance it can recognise LPS from bacteria, taxol from plant, different proteins of viral origin, Hsp 60 and 70 from the host cell itself etc. Like TLR4 other TLRs are also able to recognise a wide variety of pathogens which is briefly summarised in Table 1.

TLR	LIGANDS
TLR1	Tri-acyl lipopeptides (bacteria, mycobacteria)
	Soluble factors (Neisseria meningitides)
TLR2	Lipoprotein/lipopeptides (a variety of pathogens)
	Peptidoglycan (Gram-positive bacteria)
	Lipoteichoic acid (Gram-positive bacteria)
	Lipoarabinomannan (mycobacteria)
	A phenol-soluble modulin (Staphylococcus epidermidis)
	Glycoinositolphospholipids (Trypanosoma Cruzi)
	Glycolipids (Treponema maltophilum)
	Porins (Neisseria)
	Zymosan (fungi)
	Atypical LPS (Leptospira interrogans)
	Atypical LPS (Porphyromonas gingivalis)
	HSP70 (host)
TLR3	Double-stranded RNA (virus)
TLR4	LPS (Gram-negative bacteria)
	Taxol (plant)
	Fusion protein (RSV)
	Envelope proteins (MMTV)
	HSP60 (Chlamydia pneumoniae)
	HSP60 (host)
	HSP70 (host)
	Type III repeat extra domain A of fibronectin (host)
	Oligosaccharides of hyaluronic acid (host)
	Polysaccharide fragments of heparan sulfate (host)
	Fibrinogen (host)
TLR5	Flagellin (bacteria)
TLR6	Di-acyl lipopeptides (mycoplasma)
TLR7/8	Imidazoquinoline (synthetic compounds)
	Loxoribine (synthetic compounds)
	Bropirimine (synthetic compounds)
TLR9	CpG DNA (bacteria)
	Hemozoin (protozoa)
TLR10	?
TLR11	Component of uropathogenic bacteria (bacteria)
	Profilin like molecule (protozoa *Toxoplasma gondii*)

Table 1. TLRs and their corresponding ligands.

6. TLRs bridge innate and adaptive immunity

TLRs serve as a link between innate and adaptive immunity by induction of dendritic cell (DC) maturation and directing T helper responses (Parker et al., 2006). It has been reported that stimulation of specific TLRs leads to induction of either IL-10 or IL-12 that results in a response biased towards either Th1 or Th2 cytokines. TLR2 mediated response preferentially leads to release of Th2 cytokines while TLR4 induces Th1 cytokine release. DCs have been reported to express TLRs on their surfaces which respond to different microbial antigens differently. Immature DCs have high phagocytic activity but low T cell activation potential and these are capable of detecting, capturing and phagocytosing pathogens that ultimately leads to activation of TLRs and cytokine release. A signalling cascade commences after TLR activation (as described later) which serves as a complex differentiation programme for DCs, collectively termed DC maturation. This DC maturation is characterised by up-regulation of co-stimulatory molecules such as CD40, CD80, and CD86. CD80 and CD86 are the two requisite signals for naïve T cell activation (Banchereau & Steiman, 1998; Parker et al., 2006).

Also, when TLRs on many cell types are stimulated by TLR agonists, bacteria and viruses, it leads to the production of type I interferon (IFN-α/β) (Parker et al., 2006). This response is popularly attributed to be part of first line of defence against infection and a central modulator of adaptive immunity. Proliferation of memory T cells, inhibition of T cell apoptosis, enhanced IFN-γ secretion, B-cell isotype switching and differentiation into plasma cells and NK cell activation are some of the attributes of IFNs owing to their diverse functions in the development of adaptive immunity.

In addition to up-regulation of CD80/CD86 molecules on DCs and production of type I interferon (IFN-α/β) to control T cell activation, another mechanism of T cell activation exists in which T cell responses are regulated by $CD4^+$ $CD25^+$ suppressor or regulatory T cells (Treg cells). The Treg cells function to induce tolerance in peripheral T cells (both self-reactive and non-self-reactive T cells), a malfunctioning of these cells leads to autoimmune diseases. It has been reported that DCs produce IL-6 in response to TLR activation that is critical for T cell activation as it relieves suppression of effector T cells (non-self-reactive T cells) by Treg cells (Pasare & Metzitov, 2003). Pasare & Metzitov also report that T cell activation occurs even in the absence of IL-6 when Treg cells are removed. This suggests that induction of co-stimulatory molecules on DCs is enough for T cell activation in the absence of Treg cells.

7. TLR signalling

Toll like receptors after recognising PAMPs initiate intracellular signalling that leads to the activation of NF-κB (Nuclear factor kappa B) or IRF3 (Interferon regulating factor 3) and subsequently expression of genes under their control takes place. To induce intracellular signal transduction, TLRs either homodimerise or heterodimerise upon interaction with PAMPs. Probably there are two pathways regarding TLR signal transduction, MyD88 (Myeloid Differentiation Factor 88) dependent and MyD88 independent.

MyD88 dependent signalling pathway is found to be central to all TLRs except TLR3. TIRAP (TIR domain containing adaptor protein) is essential for MyD88 dependent signalling through TLR2 and 4 as revealed by the studies with TIRAP deficient mice.

MyD88 dependent signalling involves a number of molecules which are briefly described below.

MyD88

It is encoded by MyD88 gene (Muzio et al., 1997; Wesche et al., 1997; Burns et al., 1998). This protein is utilised by all TLRs except TLR3 as an adaptor to transmit the signal inside the cell resulting in activation of transcription factor NF-κB. Data indicates that another protein TIRAP also known as MAL (MyD88 adaptor like protein) is required by MyD88 to be recruited to TLR2 and TLR4. MyD88 protein has two domains- N terminal death domain (DD domain) and C terminal TIR domain. It interacts with the TIR domain of TLR via its C terminal TIR domain. MyD88 is also reported to interact with IL-1R, IRAK1, IRAK2, RAC1 (Ras mediated C3 botulinum toxin1) and many other proteins.

IRAK

IRAKs (IL-1R associated protein kinases) are protein kinases that act downstream of MyD88. Four IRAKs are identified in mammals- IRAK1, 2, 4 and M (Janssens & Beyaert, 2003). While IRAK1 and 4 are expressed in all cell types, IRAK2 shows narrower distribution and IRAK M is reported to be only expressed in cells of myeloid origin. IRAKs have an N terminal death domain but lack a TIR domain. But a central serine threonine kinase domain is present. IRAK1 and 4 have intrinsic kinase activity while IRAK2 and M are with no kinase activity.

TRAF

TRAFs (TNF receptor associated factors) are proteins having an N terminal coiled coil domain known as TRAF-N and a conserved C terminal domain known as TRAF- C (Bradley & Pober, 2001). There are six members in the mammalian TRAF family (TRAF1, TRAF2, TRAF3, TRAF4, TRAF5 and TRAF6). Binding of TRAF to its interacting proteins require that proteins should contain TRAF binding motif of which consensus sequence is identified and found to be as Pro-X-Glu-X-X-(aromatic/acidic residue) (Ye et al., 2002). This motif is found to present in CD40, IRAK1, IRAK2, IRAK4, TRANCER (TNF related activation induced cytokine receptor).

TAK1 and TABs

Activation of IKK complex (Inhibitor of NF-KB kinase complex) by TRAF6 requires two factors TRIKA1 (TRAF6 regulated IKK activator1) and TRIKA2 (TRAF6 regulated IKK activator2). Further studies revealed that TRIKA1 is composed of Ubc13 (Ubiquitin conjugating enzyme 13) and Uev1A (Ubiquitin conjugating enzyme variant1) which act as ubiquitin conjugating enzyme complex. Polyubiquitination of TRAF6 is done by TRIKA1 complex with lysine 63 (K63) of ubiquitin. This polyubiquitination directly activates the TAK1 in a proteasome independent manner. TRIKA2 is composed of TAK1, TAB1 and TAB2. TAK1 (TGF-β activated kinase) belongs to a MAPKKK family of protein kinases (Yamaguchi et al., 1995). TABs are TAK1 binding proteins (Shibuya et al., 1996; Takaesu et al., 2000). TAB1 acts as co-activator of TAK1 enhancing its kinase activity while TAB2 has an adaptor function linking TAK1 to TRAF6.

NF-κB

NF-κB (Nuclear factor kappa B) is a transcription factor that controls the expression of genes involved in inflammation, immunity and apoptosis. It was discovered as a transcription

factor for the K chains in immunoglobulins. About 100 genes are under the transcriptional control of NF-κB. It belongs to rel family of proteins. NF-κB is an evolutionary conserved protein having five members in mammals- p50, p52, RelA/p65, RelB and RelC. All these function either as homodimer or heterodimer for e.g. p50 homodimer and p50/p65 heterodimer. Their ability to regulate and control transcription also differs markedly, for instance p65 and Rel-C are most potent transcriptional activators while p50 homodimers seem to repress transcription. NF-κB is composed of two subunits p50 and p65. It is bound to an inhibitory protein IκB via non-covalent interaction which hampers its activity. Studies indicate that p50 and p65 dimerise around a 10 base pair region referred to as κB sites. Sequence of this site is 5'GGGRNNYYCC3' where R, Y and N refer to purine, pyrimidine and any base respectively.

7.1 MyD88 dependent signalling pathway

MyD88 is an adaptor protein that is recruited to the TIR domain of TLR upon its activation via C terminal TIR domain. MyD88 also has a death domain at its N terminal end spaced with a short linker sequence from its C terminal. MyD88 then recruits IRAK4 (IL-1R associated protein kinase 4) at its death domain via its N terminal. IRAK4 has N terminal death domain and a central serine/threonine kinase domain that is essentially required for its kinase activity and downstream signalling. This recruitment of IRAK4 to MyD88 induces conformational changes in IRAK4 that allows the interaction of IRAK1 with it and then IRAK4 acts on IRAK1 to phosphorylate it. Also upon activation, IRAK1 starts auto-phosphorylating itself (Takeda & Akira, 2005). To this assembly, TRAF6 (TNF receptor associated factor 6) further associates via phosphorylated IRAK1. TRAF6 acts as a signalling mediator for both IL-1R/TLR superfamily and TNF receptor superfamily. Association of TRAF6 with phosphorylated IRAK1 leads to the dissociation of both these mediators from the assembly and binding to TAK1 (Transforming growth factor β activated kinase), TAB1(TAK1 binding protein 1) and TAB2 (TAK1 binding protein 2). TAK1 belongs to MAPKKK (Mitogen activated protein kinase kinase kinase) family. TAB1 acts as an activator of TAK1 while TAB2 functions as an adaptor molecule linking TAK1 to TRAF6. Recently, another TAK1 binding protein, TAB3 came into being and might function similar to TAB2. This causes phosphorylation of TAB2 & TAK1 and degradation of IRAK1. This remaining complex i.e. TAK1, TAB1, TAB2 and TRAF6 now gets associated with ubiquitin ligase UBC13 (Ubiquitin conjugating enzyme 13) and UEV 1A (Ubiquitin conjugating enzyme E2 variant 1). This leads to activation of TRAF6 which in turn activates TAK1. TAK1 activation takes place through linkage of a lysine63 linked polyubiquitin chain via TRAF6-UBC13 complex where TRAF6 acts as an E3 Ubiquitin ligase (Wang et al., 2001). Activated TAK1 is responsible for the phosphorylation of MAPK and IKK complex (Inhibitor of NF-κB (IκB) kinase complex). IKK has three subunits, IKK1 or IKKα, IKK2 or IKKβ and IKKϒ or NEMO (NF-κB essential modulator) (Karin & Ben-Neriah, 2000). This kinase complex phosphorylates IκB at conserved serine residues in N terminal which mark it for ubiquitination and its subsequent degradation via proteasome. Removal of inhibitor from NF-κB leads to its activation and translocation from cytosol to nucleus where it binds to NF-κB binding regions (present in the genes under the control of NF-κB transcriptional activation) and induce the transcription of genes responsible for synthesis of effector molecules that act against the invading pathogen or PAMPs and lead to their destruction.

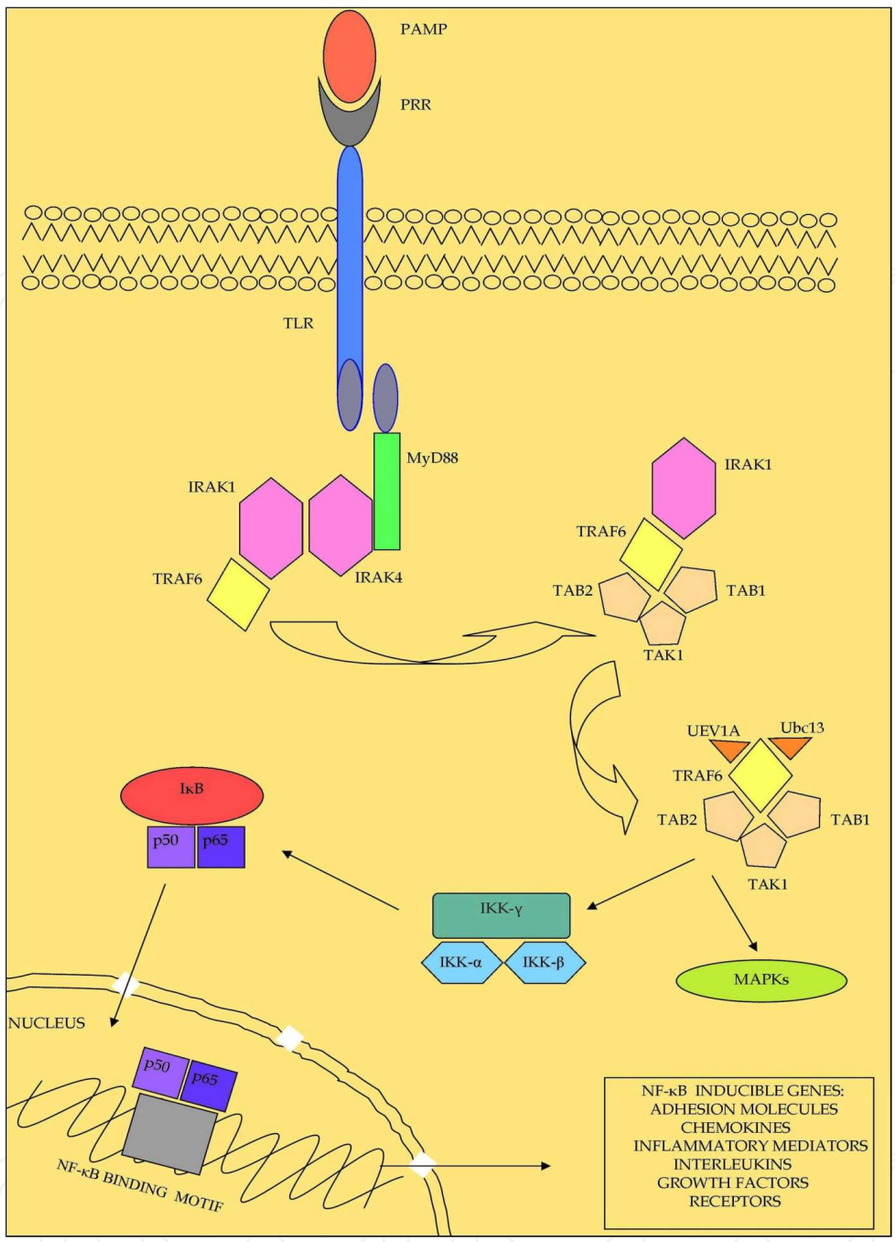


Fig. 3. Upon stimulation of TLR with suitable PAMP, MyD88 is recruited to TIR domain of TLR. IRAK4 then associates with MyD88. This causes IRAK1 to attach with IRAK4 which causes its phosphorylation. TRAF6 then joins this complex and thereafter causes IRAK1 to dissociate from IRAK4 along with it. Later TRAF6 and IRAK1 bind to TAK1, TAB1 and TAB2. Later IRAK1 is degraded and remaining complex joins Ubc13 and Uev1A which causes polyubiquitination of TRAF6 and activates TAK1 in a proteasome independent manner. Activated TAK1 phosphorylates both MAPK and IKK complex. IKK complex phosphorylates IκB that leads to its ubiquitination and then degradation in proteasome. Release of IκB from NF-κB activates it which then translocates into the nucleus and causes the expression of genes which are under its transcriptional control including genes involved in apoptosis, inflammation and immunity.

7.2 MyD88 independent signalling pathway

Studies have revealed that upon stimulation with LPS in MyD88 deficient cells, there is still production of NF-κB, although the production is delayed. This leads to the fact that TLR signalling can also occur in the absence of MyD88 i.e. independently of MyD88. TLR3 utilizes MyD88 independent signalling pathway to activate IRF3 that is responsible for the up-regulation of interferon (IFN) inducible genes and the production of IFN-β. This MyD88 independent signalling pathway utilizes another adaptor molecule known as TRIF/TICAM1 (TIR domain containing adaptor protein inducing IFN-β/ TIR domain containing molecule 1). TRIF has TRAF6 binding motifs (T6BM) in its N terminal and a TIR domain and RHIM (Receptor interacting protein 1 homotypic interaction motif) domain at its C terminal. TRIF is the only molecule meant to be involved in signalling through TLR3. Studies with TRIF deficient mice showed impaired response in activation of IRF3 and expression of IFN inducible genes only with ligands of TLR3 and TLR4 (Hoebe et al.,2003). IRF3, 5 and 7 play important roles in expression of IFN inducible genes during viral infection. IRF3 is typically required for the expression of genes encoding IFN-β and genes under the control of other interferons (Yoneyema et al., 1998). Upon activation of TLR3 with its ligand, TRIF is recruited to the TIR domain of TLR3. Then TRIF associates at its N terminus via two molecules- TRAF6 and TBK1 (TRAF family member associated NF- κB activator (TANK) binding kinase 1). TBK1 is responsible for phosphorylating the IRF3 at its C terminal regulatory domain which leads to their dimerization (Sharma et al., 2003). Dimers are then able to translocate into the nucleus and associate with co-activators p300 and CBP(cAMP responsive element binding protein).This then causes the expression of genes encoding IFN-β and other TypeI interferons (Taniguchi & Takaoka, 2002). These TypeI interferons via JAK-STAT signalling pathway are capable of inducing the expression of IFN inducible genes like GARG16 (Glucocorticoid attenuated responsive gene 16), IPG1(Immunoresponsive gene1) and CXCL10 etc. Also, IRF7 is produced later in viral infections via interferons whose expression is regulated by IRF3. TRAF6, another molecule that associates with TRIF at its N terminal is meant to activate NF-κB. TRAF6 binds to N terminal of TRIF via its TRAF-C. TRIF has three T6BM with consensus sequence Pro-X-Glu-X-X-(aromatic/acidic residue). Also, to the C terminal of TRIF, RIP1 (Receptor interacting protein 1) binds which is discovered recently and also found to activate NF- κB (Meylan et al., 2004). Activated NF-κB then is able to translocate and causes the expression of genes under its control. It has been found that transcriptional activation of IFN-β encoding gene needs both NF-κB and IRF3. However, inflammatory cytokine production still remains impaired.

Search for adaptors containing TIR domain led to the discovery of a new adaptor molecule known as TRAM/TICAM2 (TRIF related adaptor molecule/TIR domain containing molecule2) (Bin et al., 2003). Studies with TRAM deficient mice revealed that TRAM is involved in signalling through TLR4 in a MyD88 independent/TRIF dependent manner (Yamamoto et al., 2003). TRAM has a TIR domain in the C terminal and it acts upstream of TRIF while mediating TLR4 signalling exclusively. Studies showed that siRNA mediated inhibition of TRAM expression causes impairment of IRF3 activation and expression of IFN inducible genes only in response to TLR4 ligand, eg. LPS. However, TRAM knockout mice shows normal activation of IRF3 and expression of IFN inducible genes in response to TLR3 activation. Hence, TRAM is only involved in signalling through TLR4 but not TLR3. MyD88 deficient macrophages when stimulated with LPS show activation of IRF3 and also production of NF-κB, although production is delayed. Also, the production of inflammatory

cytokines is impaired in these cells. Studies with TRIF and TRAM deficient mice showed that for the production of inflammatory cytokines via TLR4, activation of both the signalling pathways is required i.e. MyD88 dependent and independent, although the mechanism is not clear. However, the production is not affected via MyD88 dependent pathway in response to ligands of TLR2, 7 and 9. Another adaptor that is involved in signalling via TLR4 is TIRAP/MAL (TIR domain containing adaptor protein/MyD88 adaptor like protein) (Horngs et al., 2001). TIRAP deficient mice show impaired production of inflammatory cytokines in response to TLR2 and 4 ligands but not to TLR3, 5, 7 and 9 ligands. This confirms its role in signalling through TLR2 and 4. TIRAP deficient mice also show IRF3 activation and production of late phase NF-κB as seen in the studies with MyD88 deficient mice. TIRAP has a C terminal TIR domain but it lacks a death domain that is present in MyD88. It acts upstream of MyD88.

LPS signalling through TLR4 is mediated with the help of several other proteins eg. MD-2 is a novel protein that mediates the TLR4 signalling in response to LPS. MD2 functions to bind LPS and then presents this LPS to TLR4 via physically interacting with it. MD2 is found to attach with TLR4 extracellular domain. Also, another protein CD14 is found to facilitate LPS signalling via TLR4. CD14 along with LBP (LPS binding protein) binds to LPS (Wright et al., 1990) and can initiate signalling via transmembrane receptors like TLR4.

7.2.1 Genes under transcriptional control of NF-κB

NF-κB is crucial for the expression of genes which are involved in immune responses (both innate and adaptive), inflammation, viral infection, stress, cytokine signalling, acute phase responses etc. Genes under the regulation of NF-κB have NF-κB binding sites in their promoter region. Adhesion molecules like ICAM-1, VCAM-1, E-selectins are expressed as a result of NF-κB transcriptional activation. ICAM-1/CD54 (Intercellular adhesion molecule -1) is expressed on endothelial and immune system cells. ICAM-1 expression upon required stimulus is enhanced via NF-κB activity. ICAM-1 binds to LFA1 (Lymphocyte function-associated antigen1), a receptor on leukocytes. Leukocytes adhere and then migrate into the tissues via ICAM-1 and LFA-1 interaction and carry out the required actions. VCAM-1(Vascular cell adhesion protein-1) is present in the endothelial cells and function to adhere lymphocytes, basophils, monocytes etc. to vascular endothelium. E-selectin/CD62E/ELAM-1(Endothelial leukocyte adhesion molecule-1) is expressed on vascular endothelium in response to TNF-α and IL-1β. It binds to carbohydrate moieties on some leukocytes. Genes expressing growth factors are also up-regulated by NF-κB like genes for GM-CSF (Granulocyte macrophage colony stimulating factor), M-CSF/CSF1 (Macrophage colony stimulating factor/ Colony stimulating factor3) and G-CSF(Granulocyte colony stimulating factor/ Colony stimulating factor 3). GM-CSF is a cytokine that stimulates stem cells to differentiate into neutrophils, eosinophils, basophils, granulocytes and monocytes. M-CSF is also a cytokine that stimulates stem cells to differentiate into macrophages and related cell types. G-CSF acts on bone marrow to form more of granulocytes and stem cells. Various chemokine genes also show enhanced expression in response to NF-κB like genes for Eotaxin, RANTES (Regulated upon activation, normal T cell expressed and secreted), MIP 1α (Macrophage inflammatory protein 1 α) etc. Eotaxin is a chemokine that recruits eosinophils while RANTES is a chemoattractor for T cells, K cells, eosinophils, dendritic cells and is also responsible for recruiting leukocytes at inflammatory sites. Several cell surface receptor genes are up-regulated eg. CCR5 (C-C chemokine receptor

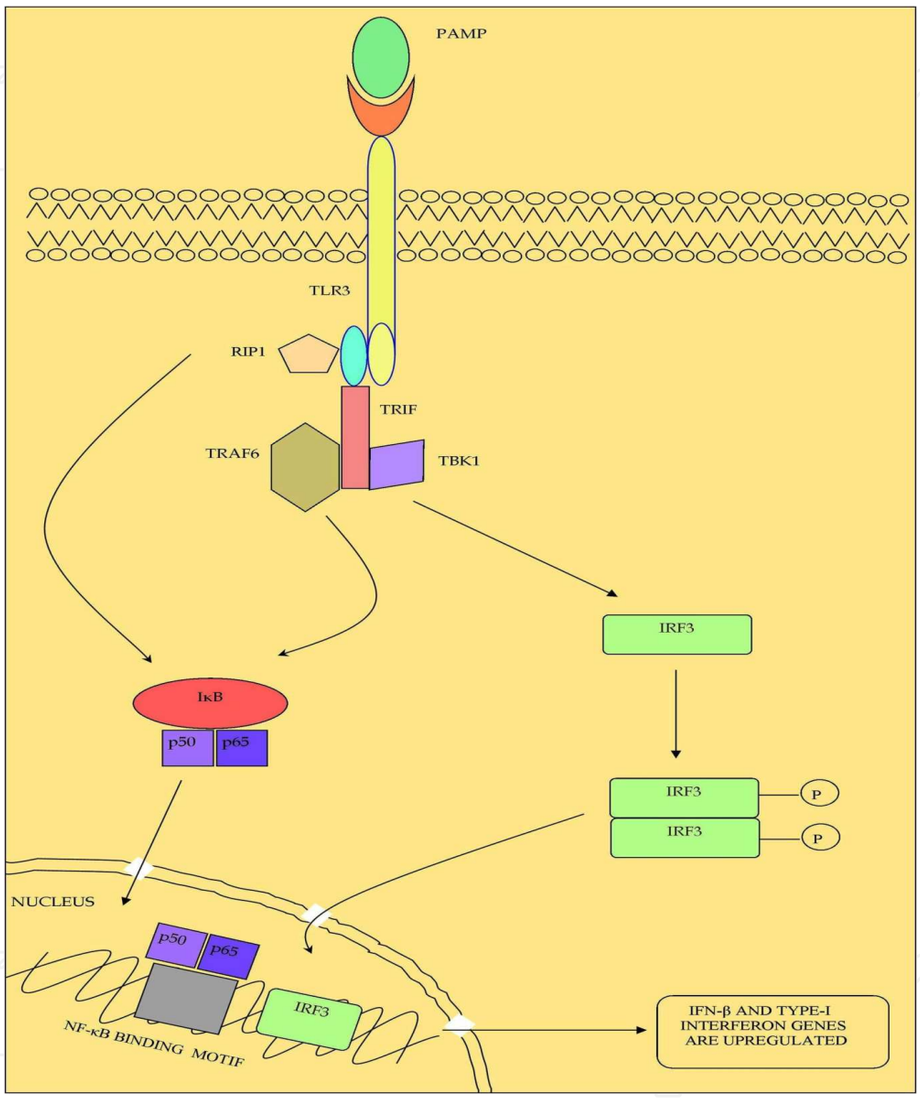


Fig. 4. TLR3 recruits TRIF upon stimulation with its ligand. To N terminal of TRIF, TBK1 associates which carries out later phosphorylation of IRF3. Upon phosphorylation, IRF3 forms dimer and translocates into nucleus and causes the expression of genes of IFN-β and Type I interferons. Also, to the N terminal of TRIF, TRAF6 binds which activates NF-κB which is then able to translocate and causes the transcription of genes under its control. Genes encoding IFN-β require activation of both NF-κB and IRF3.

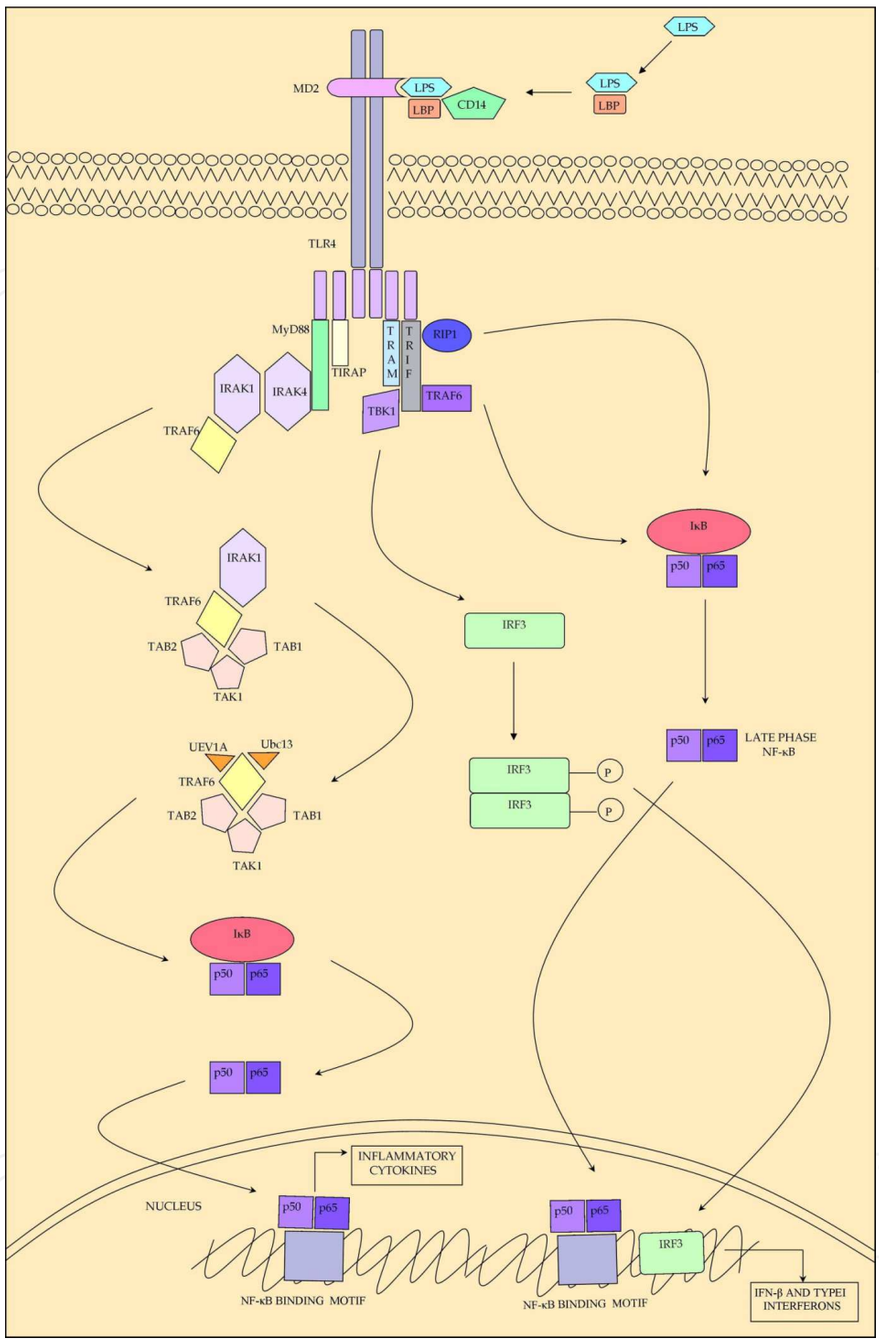


Fig. 5. Response to LPS is mediated through TLR4; LPS binds to LBP, and then forms a complex with CD14. This complex interacts with MD-2 which is able to interact physically with TLR4. Signalling downstream afterwards proceeds either via MyD88 dependant or MyD88 independent pathway. In MyD88 dependant pathway, NFκB is activated which induces transcription of several genes including genes of inflammatory cytokines. On the other hand, in TIRAP and MyD88 knockout mice, activation of IRF3 and late phase NFκB takes place both of which are able to initiate transcription of the genes under their respective control.

type 5), CD86, TCR(T cell receptor), MHC class I & II, PAF(Platelet activating factor) receptor. Enhanced expression of MHC molecules and TCR means to up-regulate T cell activation and hence adaptive immunity. CD80 and CD86 are the major T cell co-stimulatory molecules. CCR5 is the receptor for RANTES, MIP 1α and 1β. Cytokine IL-1β, IL-2, IL-6 and TNFα show enhanced expression after NF-κB activation. NF-κB dependent stimulation of iNOS promoters also takes place.

8. TLR signalling is negatively regulated

Docking of pathogen onto TLRs and their subsequent stimulation induces production of inflammatory cytokines such as TNF-a, IL-6 and IL-12. An uncontrolled and excessive cytokine production can lead to manifestation of serious autoimmune and inflammatory diseases. Hence, to avoid an excessive inflammatory response, organisms have evolved mechanisms which make a balance between TLR activation and inactivation. Several molecules modulating TLR-mediated responses have been unravelled. Negative regulators of TLRs can either be extracellular or intracellular (Arancibia et al., 2007).

Extracellular regulators comprise of soluble form of TLRs. Soluble form of TLR2, sTLR2, is produced by a post-translational modification of the membrane bound TLR2. It is reported that if sTLR2 splice variant expression is inhibited, an augmented response to bacterial lipopeptide is seen (Lebouder et al., 2003). Alternate splice variant of TLR4 (soluble TLR4), sTLR4, is shown to be involved in the inhibition of LPS-mediated TNFα production and NFκB activation, blocking MD-2 (a co-receptor of TLR4) recruitment to the TLR4-CD14 complex. Also, soluble product of TLR5, sTLR5, is seen to be implicated in cellular response of flagellin that induces an increased NF-κB activation by an unknown cellular mechanism.

A variety of intracellular molecules (adaptors and kinases) are found to regulate TLR signalling. An alternatively spliced variant of MyD88 that lacks the intermediary domain of MyD88 (MyD88s) is induced in monocytes upon LPS stimulation. Overexpression of MyD88s results in impaired LPS-induced NF-κB activation through inhibition of IRAK-4-mediated IRAK-1 phosphorylation. IRAK-M is another negative regulator of TLR signalling cascade and it lacks catalytic kinase activity. IRAK-M inhibits expression of pro-inflammatory cytokines by preventing IRAK-1/IRAK-4 dissociation from MyD88, hence causing inhibition of IRAK-1-TRAF6 complex formation. The fact that IRAK-M plays a crucial role in regulating MyD-88 dependant signalling pathway can be established from the information that IRAK-M-/- mice overproduce inflammatory cytokines in response to LPS and CpG DNA (Arancibia et al., 2007).

A protein associated with Toll-Like Receptor 4 (PRAT4A) regulates cell surface expression of TLR4. PRAT4A is associated with the immature form of TLR4 but not with MD-2 (a TLR4 co-receptor) or TLR2. PRAT4A knockdown led to the profound defect in LPS responsiveness in a cell line expressing TLR4/MD-2, probably due to impaired maturation of TLR4, leading to the lack of mature TLR4/MD-2 on the cell surface. PRAT4A is likely to be a component of the machinery facilitating TLR4/MD-2 trafficking to the cell surface. Hence, PRAT4A is another negative regulator of TLR4.

SOCS1 and SOCS3 belong to SOCS (Suppressor of cytokine signaling) family of proteins. These proteins themselves induced by cytokines, negatively regulate TLR4/NFκB signalling pathways (Gingras et al. 2004). In SOCS 1-/- mice defective induction of LPS tolerance was

observed as they were found to be hypersensitive to LPS-endotoxin. In the same manner, LPS induced TNF-α production was found to be suppressed in macrophages exposed to IL-10 and IL-6 isolated from SOCS3-/-(Arancibia et al., 2007).

PI3K, implicated in TLR signalling, has been found to suppress both MAPKs and NFκB induced by LPS, thereby decreasing TNF-α production (Arancibia et al., 2007). Tollip (Toll interacting protein) has also been found to play an inhibitory role in TLR signalling. Tollip when in association with TIR domain decreases IRAK-1 phosphorylation upon LPS activation. A plausible role of PI3K in regulating inhibitory effects of Tollip has been proposed. PI3K does that by interacting with 3' phosphorylated phosphatidylinositides (Arancibia et al., 2007).

SIGIRR (single immunoglobulin IL-1 receptor-related molecule) and T1/ST2, membrane bound proteins adhered to the TIR domain, have also been found to be negative regulators of TLR signalling (Takeda & Akira, 2005). Nucleotide oligomerization domain receptor (NOD2), a mammalian PRR, too seems to be a negative regulator as NOD2-/- macrophages when stimulated by TLR agonists produce significantly higher amount of cytokines but on restoration of NOD phenotype, cytokine expression is lowered (Arancibia et al., 2007).

Activating Transcription Factor-3 (ATF-3) has also been found to negatively regulate TLR-signalling pathways (Whitmore et al., 2007). It has been observed that different TLR ligands (i.e., zymosan for TLR2/3, pIC for TLR3, LPS for TLR4, and CpG-ODN for TLR9) stimulate rapid induction of ATF3 in cultured mouse macrophages. It is reported that primary macrophages of mice lacking *atf3* gene (ATF3-knockout (KO)) show enhanced expression of TLR-induced IL-12 and IL-6 when compared to wild type macrophages. In a reporter assay, ectopic expression of ATF3 was found to antagonize TLR-stimulated IL-12p40 activation. Further, CpG oligodeoxynucleotide, a TLR9 agonist when introduced in ATF3-KO mice resulted in enhanced cytokine production from splenocytes. Hence, it can be concluded that *atf3* deficiency leads to altered pattern of immunological response and ATF-3 is a negative regulator of TLR pathways.

In addition to these, degradation of TLRs (either ubiquitination mediated or lysosomal) is also proposed as a mechanism for negatively regulating TLR signaling (Takeda & Akira, 2005; Wang et. al., 2007). A RING finger protein, Triad3A, has been found to act as an E3 ubiquitin ligase, ligating ubiquitin molecules onto the TLR4 and TLR9 and enhancing their proteolytic degradation. A recent study by Wang et. al. (Wang et. al., 2007) reveals another mechanism of negative regulation of TLR4 signalling, by lysosomal degradation of TLRs. They propose that Rab7b, a lysosome associated small GTPase negatively regulates NF-κB and IRF3 signalling pathways in macrophages by promoting lysosomal degradation of TLR4 and decreasing the cell surface expression of TLR4. These complex mechanisms of negative regulation of TLRs emphasize that it is important for prevention of uncontrolled immune activation in the host.

9. TLRs in various diseases

9.1 TLRs in nervous system diseases

TLRs have been considered earlier as receptors expressed solely on antigen presenting cells of the immune system i.e. B cells, dendritic cells, monocytes, macrophages etc. and mediate

innate immunity. However, with advancement in techniques, it is clear that nearly all cells within the body express TLRs, including different brain cell types such as microglial cells, astrocytes, oligodendrocytes and neurons within the CNS (Central Nervous System). The present section will focus the role of TLRs in these brain cells.

Microglia

Microglial cells are bone marrow-derived macrophage-like cells constituting about 10% of the adult CNS and mediate neuronal immune interactions under both physiological and pathological conditions (Pessac et al., 2001). Microglial cells are the key defence against invading pathogens within the CNS, and it is not surprising, therefore, that activation of these cells either by a single type of ligand or a combination of ligands, leads to secretion of a milieu of cytokines and chemokines. It is now well known that microglial cells express wide receptors of TLRs in addition to their adapter proteins, required for functional downstream TLR signalling. Recent studies showed that TLR1-9 are expressed in microglia (Jack et al., 2005). Upon activation, TLR mRNA and protein expression is increased in microglia. As a result of TLR activation, these cells secrete higher amounts of pro-inflammatory cytokines and hence show pathogen specific responses. TLR signalling in microglia may also have a role in cell death and survival following inflammatory activation which suggests a paradigm in which auto-regulation of the innate immune system exists in the CNS which helps to prevent excessive inflammation during pathogen infection (Jack et al., 2007; Tanaka et al., 2008; Okun et al., 2009).

Astrocytes

Astrocytes are characteristic star-shaped glial cells in the brain and spinal cord and perform many functions, including biochemical support to endothelial cells that form the blood–brain barrier provision of nutrients to the nervous tissue, maintenance of extracellular ion balance, and a role in the repair and scarring process of the brain and spinal cord following traumatic injuries. Similar to microglial cells, astrocytes exhibit a wide expression of TLRs. Astrocytes express robust TLR 3 with low expression of TLR 1, TLR4, TLR5 and TLR9, and with rare expression of TLR2, TLR6, TLR7, TLR8 and TLR10. Several cytokines and chemokines are reportedly produced following TLR activation in astrocytes. TLR 3 signalling induces strongest pro-inflammation polarizing response by secreting increased levels of IL-12, TNF-alpha, IL-6, CXCL-10, IFN-beta and IL-10 (Jack et al., 2005). Both cytokines and TLR agonists induce expression of chemokine ligands i.e. CCL2, CCL3, CCL5, ICAM-1 and vascular cell adhesion molecule-1 (VCAM-1) (Carpentier et al., 2005 ; Okun et al., 2009).

Oligodendrocytes

Oligodendrocytes are a type of neuroglia and function as an insulation of axons in the CNS. As compared to other CNS cell types, very little is known regarding the expression and function of TLRs in oligodendrocytes. The first report on TLRs in these cells has shown the predominant expression of TLR2 and 3 as evidenced by promotion of survival, differentiation, and myelin-like membrane formation and induction of apoptosis by TLR2 agonist, zymosan and TLR3 agonist, poly-I:C respectively (Bsibsi et al., 2002). While the exact role of TLR2 in oligodendrocytes is unknown, *in vivo* evidences suggest that activation of this receptor is involved in CNS repair by enhancing myelination of neurons in the CNS and damage repair after spinal cord injury (Okun et al., 2009).

Neurons

Neurons are the core components of the CNS which processes and transmits information by electrical and chemical signalling. During the past few years, evidence for the neuronal expression of TLRs has increased, suggesting a role for this receptor family in neurons during physiological as well as pathological conditions. The expression of the mRNA for TLRs1-9 as well as protein levels of TLR 2, 3 and 4 has been shown *in vivo* following infection in a parasitic model of neurocysticercosis (Tang et al., 2007). Studies provide evidence that neurons from both the central and peripheral nervous systems express TLR3 and that it is concentrated at the growth cones of neurons. In addition to TLR expression in brain diseases, it is known that neuronal TLR activation plays a role in development. It has been reported recently that treatment of cultured embryonic cortical neurospheres with a TLR3 ligand significantly reduced proliferating (BrdU-labeled) cells and neurosphere formation, whereas neural progenitor cells (NPC) from TLR3-deficient embryos formed greater numbers of neurospheres compared to neurospheres from wild-type embryos (Okun et al., 2009). A distinct difference is apparent between the effects of TLR activation in differentiated neurons and neuronal progenitor cells. Apart from the classical TLR ligands such as LPS (TLR4) or Pam3CSK4 (TLR2), it is considered that neuronal TLRs respond to endogenous ligands but not to pathogen-derived ligands (Okun et al., 2009).

9.1.1 TLRs in neurodegeneration

TLRs generally respond against invading pathogens, however, they can also be activated in the absence of microbial infection and regulate neurogenesis. Studies examining inflammatory markers in normal brain aging have also suggested a dynamic regulation of TLRs and hence, showed its participation in aging and age-related disease. Despite the emerging role of TLRs in stokes, AD (Alzheimer's disease) and MS (Multiple sclerosis), very little is known regarding the function of these receptors in other neurodegenerative disorders. In this context, the role of TLRs in brain diseases such as, Alzheimer's disease, multiple sclerosis and other neurodegenerative conditions is discussed herewith.

9.1.2 TLRs in Alzheimer's disease

Alzheimer's disease (AD) is a progressive neurodegenerative disease characterized by gradual onset and advancement of memory loss and other cognitive deficits. Definitive diagnosis of AD is based on the presence of extracellular amyloid plaques comprised of neurotoxic amyloid β-peptide (Aβ) which is generated by proteolysis of the β-amyloid precursor protein (APP), and intracellular neurofibrillary tangles composed of hyper-phosphorylated insoluble forms of *tau* protein. TLR expression is up-regulated and increased in the AD brain. A screening of TLRs in murine models of AD revealed an up-regulation of TLR2 and TLR7 transcription levels compared to wild-type controls. Further, multiple TLR genes (1-8) are expressed in microglia in post-mortem tissue from AD patients, with varying levels of expression. The increased expression of TLRs in AD positions them as potential players in neurodegenerative mechanisms and disease progression. The TLR4 gene has emerged as a candidate susceptibility gene for AD. A common missense polymorphism occurs at the TLR4 gene locus resulting from an adenine to guanine substitution 896 nucleotides downstream of the transcription start site. This substitution causes the replacement of glycine for aspartic acid at amino acid 299 (Asp299Gly), and alters

the structure of the extracellular domain of TLR4. This mutation attenuates TLR4 signalling in response to LPS and diminishes the ability to induce inflammation (Arbour et al, 2000). In AD brain, activated glia expressing high levels of TLR4 and TLR2 surround Aβ plaques. The close association between Aβ plaques and reactive astrocytes and microglia has led to the assertion that these cells contribute to plaque formation (Walter et al., 2007). TLR4 expression increases during exposure to Aβ and the lipid peroxidation product 4-hydroxy-nonenal (HNE). Further, c-Jun N-terminal kinases (JNK) and caspase-3 activity levels are augmented in neurons exposed to Aβ and HNE. Selective elimination of TLR4 function significantly suppresses the abilities of Aβ and HNE to induce activation of JNK and caspase-3 (Tang et al., 2007) suggesting that TLR4 expression increases neuronal vulnerability to Aβ-induced damage (Okun et al., 2009). Neurons expressing TLR4 have increased sensitivity to Aβ and are vulnerable to degeneration in AD. In addition to epidemiological studies that suggest mutations in TLR4 lead to decreased susceptibility to neurodegeneration, several data indicate that activation of TLR4 is required for clearance of Aβ in AD. In addition to TLR4, activation of other TLRs may also contribute to Aβ clearance. Whereas TLRs are activated by exogenous pathogens, mounting evidence indicates that Aβ itself activates TLRs and mediates microglial activation. At present, it still remains to be determined if the activation of TLRs by Aβ contributes to and/or inhibits AD progression. Contrasting data exist on the precise role of TLRs in Aβ deposition. Therefore, there may be a balance of TLR activation in which mild activation is beneficial, promoting Aβ uptake and breakdown. However, excessive activation of TLRs on microglia may lead to the accumulation of cytotoxic compounds such as reactive oxygen species, cytokines, complements and proteases causing damage and eventual neuronal loss. TLR signalling pathways are a potential therapeutic target in AD; however more work remains to delineate the complex interaction of TLRs in Aβ deposition and clearance and its precise role in AD development (Okun et al., 2009; Akiyama et al., 2000).

9.1.3 TLRs in Multiple Sclerosis

Multiple Sclerosis (MS) is a chronic inflammatory and demyelinating disease of the CNS and characterized by recurrent neurological dysfunction. It is believed to be an immune-mediated disease in which auto-reactive T cells enter the CNS and drive a pro-inflammatory reaction resulting in tissue injury after infection. There is a marked increase in TLR expression in multiple sclerosis lesions. Microglial cells from MS patients express TLRs 1-8, while, healthy white matter from patients does not contain TLRs. Examination of TLR3 and TLR4 localization revealed that early active MS lesions are associated with vesicular localization of TLR3 and TLR4 within microglia, located near blood vessels at the outer edges of lesions. In contrast, late active lesions also contain astrocytes bearing surface TLR3 and TLR4. This suggests that early lesions are characterized by microglia infiltration, while astrocytes are also active in later MS lesions. Researchers showed that TLR expression is up-regulated in the brain and spinal cord in animal models of MS. The exact role and mechanism of TLRs and its activation in these lesions is still unclear. One hypothesis asserts that in response to pro-inflammatory cytokines, microglial cells are capable of serving as antigen-presenting cells which can activate CD4+ T cells and facilitate neuroinflammation. Therefore, TLR activation may be an essential step in converting microglia to antigen presenting cells and facilitate T cell infiltration of MS lesions. Alternatively, TLRs may induce production of pro-inflammatory cytokines and thereby inflict damage. It can also

happen that endogenous ligands like ganglioside and sialic acid containing glycosphingolipids released from apoptotic neurons may bind to TLR4 present on microglia and activate it resulting in either neurodegeneration by releasing pro-inflammatory molecules or provide neuroprotection by attracting oligodendrocyte progenitor cells to lesion sites in MS to promote remyelination. Although TLRs often recognize pathogen-associated molecular patterns and protect the body from invasion of microbial pathogens, the expression of TLRs within multiple sclerosis suggests novel roles for these receptors in mediating neurological disease and hence can be used as a biomarker of the neurodegenerative disorders (Okun et al., 2009). Moreover, it is important to determine the precise role of distinct TLRs in Aβ recognition and clearance, and the activation of glial cells. This may open a window of hope (Arroyo et al, 2011).

9.1.4 Therapeutic approaches

TLRs are not only activated in response to microbial infection, but are critically involved in mediating neurological dysfunction. The extensive involvement of TLRs in neurodegenerative disorders provides wide opportunity for promoting and inhibiting their signalling to intervene the progression of the disease. However, it may be difficult to achieve the correct balance and appropriate timing of such interventions. There is huge variation in the TLR expression and hence the modifications will be varied across different disorders and there may exist variability within patients of the same disease. Proper TLR targeting will require extensive understanding of the pathways, mechanism activated, cell-specific responses and the course of disease progression. Both human and animal studies implicating TLRs in neural degeneration suggest direct modulation of TLR signalling as an ideal therapy. Specific strategies are necessary to circumvent this barrier and allow administration of TLR treatments to the CNS. Targeting TLRs in neurological disease will not be without difficulties. One potential obstacle to targeting TLR signalling in disease is that virtually all cells in the body express TLRs. If chronic administration of TLR agonists is necessary, it may result in overstimulation of the immune system, which limits dosage capability as well as frequency of application. CNS- specific isoforms of TLR agonists which possess high selectivity could prevent such overstimulation of peripheral immune responses. In addition, partial agonists may be useful in preventing overstimulation of TLRs in the same tissues. Another potential hurdle to TLR- directed therapeutics is cross-talk between receptor subtypes. Alternatively, specific TLR activation can induce tolerance to stimulation for other TLRs sharing same cascade. Therefore, the consequences of targeted TLR stimulation on similar signalling pathways must be carefully considered while adapting the therapeutic approaches (Okun et al., 2009).

9.2 TLRs in cancer and anti-cancer immunotherapy

Tumour cells in order to survive try to modulate their microenvironment by providing signals for uncontrolled growth, anti-apoptosis, angiogenesis and metastasis. Despite all these efforts tumour cells get noticed by the immune system which treat cancer cells as foreign. The studies have shown that tumour cells have devised sets of strategies to escape the surveillance by immune system. TLRs were earlier thought associated only with immune cells but recent findings have suggested that tumour cells too have TLRs on their surface and may play important role in tumour growth and immune surveillance escape.

The tumour cells are able to escape the immune surveillance probably by inhibitory cytokines, inflammatory factors, proteinases, and other small molecules such as nitric oxide, IL-6 and IL-12. These molecules in conjugation with TLRs may play role in development of cancer by providing resistance to tumour cells to apoptosis and immune surveillance. It has been seen that upon activation of TLR4, level of X-linked inhibitor of apoptosis and phosphorylated Akt (Protein Kinase B, PKB) are increased. Apoptosis inhibition has been seen as the case also in lymphoma and lung cancer cells. Previous studies have given evidence in support of LPS as tumour growth promoter through the NF-κB resulting in up-regulation of iNOS and MMP2 and the β1 integrin subunit (Harmey et al, 2002; Wang et al, 2003). Apart from microbial origin ligands for TLRs, the source of endogenous ligands which may promote tumour growth is not clear. The answer to the endogenous source of ligands may not only provide some insight to tumour growth but may also help to understand autoimmune diseases. It has been proposed that the TLR-4-MyD88 signalling pathway may be a risk factor for developing cancer and may represent a novel target for the development of bio-modulators. Heat shock proteins such as Hsp60, Hsp70 and Hsp90 may induce the production of pro-inflammatory cytokines such as TNF-α, IL-1, IL-6 and IL-12, release of NO and chemokines by monocytes, macrophages and dendritic cells (Neill., 2008; Asea et al., 2000; Kol et al., 2000; Singh-Jasuja et al., 2000) . There are strong possibilities of Hsp60, Hsp70 and Hsp90 being putative endogenous ligands for TLR4. Ulcerative colitis, a chronic inflammatory disease of the colon may put an individual at risk of colorectal carcinoma. Chronic hepatitis and cirrhosis, pose a risk for the development of hepatocellular carcinoma. Research in the past few years have given strong evidence that an inflammatory profile of cytokines and chemokines persisting at a particular site would lead to the development of a chronic disease. The innate immune system may give in to the promotion of tumour growth through inflammation-dependent mechanisms. Recognition of molecules either of viral or bacterial origin bearing molecular signature or pattern by TLRs on immune cells may induce an inflammatory response associated with tumour promotion. It has been observed that bacterial infection post-surgery may promote metastasis of previously dormant tumour, and LPS have implicated in leading to this situation (Hsu et al, 2011). The MyD88-independent TLR signalling involves the activation of the late phase of NF-κB in addition to the activation of IFN regulatory factor 3, which ultimately leads to the production of type I IFN (IFN a/h), IFN-inducible gene products, and an immune regulatory response. Activated TLRs on the surface of tumour cells not only promote their own proliferation but also help to build resistance to apoptosis. Further, rouge TLRs may enhance tumour cell invasion and metastasis by regulating metalloproteinases and integrins. Moreover, the control of TLRs may also lie beyond the traditional boundaries of protein molecules into world of miRNA, and their role is still being uncovered. In fact, the discovery of miRNAs has indeed brought a paradigm shift in our understanding towards the eukaryotic gene regulation. Their uniqueness lies in the fact that these molecules show cell or tissue specific expression. In principle, the miRNAs fine tune the gene expression, and similar to the classical oncogenes and tumour suppressor genes, miRNA may play part in promotion or suppression of malignancies. They act mainly by inhibiting the translation or by promoting the degradation of mRNA. For example, miRNAs like miR-146, which targets two proteins involved in TLR signalling, TRAF6 and IRAK1, negatively regulates mRNAs of both TRAF6 and IRAK1 proteins whereas its own level gets up-regulated in response to LPS. Another miRNA, miR-155 targets Src domain containing inositol 5-phophate 1(SHIP-1) and negatively regulates NF-κB signalling. Owing to their role of fine

tuner of gene expression pattern, the administration of single miRNA may affect the expression pattern of the target gene. Despite some apprehensions over the safety and efficacy of miRNA based therapies, a judicial extrapolation to TLR regulated miRNAs may provide some therapeutic solution. Also the role of innate immune system in cancer development is being looked into more seriously.

TLRs, tumour cells and Treg cells have been linked. TLR agonists can induce differentiation, proliferation or activation of Treg cells. Several TLR agonists such as Streptococcal agent OK-432, double stranded RNA and CpG DNA have anti-tumour activity (Chen & Oppenheim, 2009). TLR agonists overcome tolerance to self-antigens or tumour-antigens by directly or indirectly relieving suppression of effector T cells by Treg cells. A TLR2 agonist has been reported to transiently suppress FoxP3 (a member of forkhead/winged helix family of transcription factors and a master regulator of Treg development) expression and render resistance to suppression by Treg cells of $CD4^+CD25^+$ effector T cells. Treg cells express TLR4, 5, 7 and 8 in mice. It has been reported that transfer of Treg cells enhanced tumour growth in mice but it was reversed upon stimulation of Treg cells with a TLR8 ligand. Administration of LPS also abrogates Treg activity reveals latent anti-tumour immunity ((Chen & Oppenheim, 2009). The biggest problem in using TLRs as anti-cancer targets lies in the fact that many cancer patients have very low immunity because of anti-cancer therapy side effect, thus it becomes very difficult to get innate immune response. The quest to arrive at a point where innate immune system's stimulatory compounds are used along with anticancer agents may bear some fruit. The TLR3 has been shown to be receptor for viral dsRNA, and also seems to be potentially promising in anti-cancer therapy. Reports have shown that cancer cells themselves express TLR3 *in vivo* and agonist ploy (I:C) is activating the signalling pathway leading to the anticancer effects (Elizabeth et al., 2010; O'Neill et al., 2011). Hence, this complex relationship of tumour cells and TLRs seems to be crucial to determine the balance between beneficial and pathological roles of TLRs.

9.3 TLRs in asthma and allergy

Lungs are continuously exposed to microbial pathogens because of their constant relationship with the surrounding environment. Therefore, innate immune response in lungs to eliminate the pathogen requires expression of TLRs which would be activated upon pathogen exposure and subsequently commencing in signalling cascade. This signalling culminates in the elevated expression of IL-1-β, TNF-α, IL-12, and IFN-γ. The nature and intensity of response is regulated by the display of polarized cytokine profiles, either Th1 or Th2. Th2 cytokines are reported to play a crucial role in initiation and perpetuation of allergy and asthma (Bauer et al., 2007). Exposure to low doses of LPS results in Th2 biased response leading to onset of allergic response. On the contrary, high doses of LPS show protective effect. This is confirmed by the studies revealing an inverse relationship between allergy and asthma and early childhood exposure to rural farm environment. Although the exact mechanistic pathway is yet to be explored, but it becomes quite evident that LPS-TLR4 complex can either protect or aggravate the severity of asthma, depending on the timing of the LPS exposure. This is supported by "hygiene hypothesis" that favours that development of asthma and allergies is executed by the reduction in microbial exposure in early childhood and decrease in naturally occurring infections. Asthma and allergies are found to be less prevalent among individuals brought up in rural farm areas in their childhood (Gehring et al., 2002). Such early exposure to farms and barns or early exposure to microbes

and microbial components is attributed to the protection rendered in development of allergies in later life. This might be because of the induction of regulatory T cells that down-regulate the adaptive immune responses.

Apart from the role of TLR4 in allergies, recently it has been reported that TLR2 too modulates the development of allergic disease (Bauer et al., 2007). Studies conducted on children of farmers of Germany who have decreased risk of developing allergies were found to have augmented expression of TLR2 mRNA. The presence of asthma and allergies in the children of farmers was co-related with the genetic variation in TLR2. It was found that asthma and atopy were less prevalent in the children carrying T allele in TLR2/-16934 (Chen et al., 2007).

9.3.1 TLRs, regulatory T cells and allergy

A few studies recently have sparked widespread interest in the regulation of allergy as they claim that control of allergy is not only restricted to Th1/Th2 bias but other mechanisms as well are responsible for controlling inflammatory response and regulatory T cells (Treg cells) play a pivotal role in this regulation (Akbari et al., 2002). TLRs are expressed on Treg cells (Bauer et al., 2007). A study shows that in adults allergic to pollen, a significant reduction in the number of Treg cells and their capacity to restrain allergic response occurs when compared to healthy controls. Another finding supports that Treg cells can block allergic responses by demonstrating that activation of TLR4 expressed on the $CD4^+CD25^+$ subset of Treg cells in response to high doses of LPS may prevent activation of pathogenic T effector cells and airway inflammation and hyper-reactivity can be overcome by $CD4^+CD25^+$ Treg cell function in IL-10 dependant manner (Chen et al., 2007). Recently, a report elaborates a link between TLR2 and Treg cells as well because in TLR2 mice, $CD4^+CD25^+$ Treg cell subset was found to be significantly reduced when compared to wild type mice (Sutmuller et al., 2006).

9.3.2 Therapeutic potential of TLR ligands in allergy

The discovery that TLR signalling culminates in the activation of DCs leading to increased Th1 bias can also be applied in the treatment of allergic diseases. Particularly, TLR9 stimulation by un-methylated CpG-motif that promotes a Th1 response has been explored as potential treatment for atopic diseases like asthma and allergic rhinitis (Horner et al., 2001). In mice sensitized with allergen, CpG administration has been shown to inhibit the development of airway hyper-responsiveness and eosinophilia. It has also been demonstrated that CpG-DNA when conjugated to allergen offer a new anti-allergic strategy in which the complexes so formed show a more promising result by augmenting the immunotherapeutic effect when compared CpG-DNA given alone or CpG-DNA given mixed to allergen (Tighe et al., 2000; Horner et al., 2001). These studies encourage usage of selectively targeted allergen TLR-fusion proteins for manipulating and eliciting specific immune responses and studies are also suggestive that CpG-DNA might be a valuable and potent agent for treatment of allergies. Interestingly, imidazoquinoline resiquimod (R-848), a ligand for TLR8 has the potential to revert Th2 allergic response to Th1 because of its exceptional capability to induce Th1 response

(Hemmi et al., 2002). Hence, ligands like imidazoquinoline resiquimod too can be therapeutic targets for allergic reactions.

9.4 TLRs in autoimmune and inflammatory diseases

9.4.1 Systemic Lupus Erythematosus (SLE)

SLE is an autoimmune disorder in which antibodies are directed against a range of self-antigens. Out of these, autoantibodies to nuclear antigens are of keen importance to the clinical diagnosis of SLE. Nuclear antigens include dsDNA, ssDNA, nucleolar RNA, histone proteins and others. Emerging researches have revealed the involvement of TLRs in the progression of autoimmune diseases like SLE, rheumatoid arthritis, diabetes mellitus etc. TLR7 and TLR9 are of particular interest in the studies of SLE which are located in the endosomal compartments (Anders, 2005; Christensen et al., 2005). It has been seen that unregulated or misregulated activation of TLRs can lead to an autoimmune phenotypic appearance. Nucleic acids which are usually not immunogenic are not able to induce an immune response, but these can become immunogenic via several chemical modifications like hypo-methylation, increased oxidation and high CpG content. Immune complexes having DNA or RNA which are formed as a consequence of necrosis thus are capable of activating TLRs. It has been reported that DNA found in immune complexes has 5-6 times more of CpG content and is hypo-methylated in SLE patients. Release of autoantibodies and inflammatory cytokines which are responsible for chronic inflammation (Christensen et al., 2007; Savarese et al., 2008) can be traced to the improper activation of TLR7. In SLE patients, levels of IFNα and TypeI interferons are excessively high and it is found that higher levels of IFNα are beneficiary to the disease progression. Nowadays, TLR signalling pathways are directed for therapeutic intervention. Various key molecules of TLR7 and TLR9 signalling pathways are targeted to block downstream signalling and hence the effector responses. Molecules targeted are MyD88, TRAF6, IRAK1 & 4. Other approaches are monoclonal antibodies directed against IFNα. Also immunoregulatory DNA sequences (IRS) bind to TLRs and block their activation. Hence, these can be used as effective strategies in reduction of SLE progression molecules. Research for absolute treatment is still in its early stages.

9.4.2 Rheumatoid Arthiritis (RA)

RA is an autoimmune disorder which affects the joints most severely. RA is caused due to generation of autoantibodies against the Fc region of IgG. Usually these autoantibodies are of IgM type and referred to as rheumatoid factors. Recent studies have revealed that TLRs play a significant role in the development of RA. TLR2 & 4 seems to play a crucial role in RA. TLR2 & 4 over expression is found in the blood monocytes, synovial fluid macrophages and fibroblasts in RA (Iwahashi et al., 2004). Patients with RA are found to have presence of TLR ligands in the joints synovial fluid. These ligands can be endogenous (Heat shock proteins, HMGB1, hyaluronan etc) (Huang et al., 2009) or can be exogenous like peptidoglycan. It has been seen that MyD88 and TLR2/4 deficient mice show reduced severity of RA. Currently various approaches are investigated to treat RA effectively. TLR antagonists and various TLR signalling molecules are targeted as a promising agent for treating RA.

9.4.3 Inflammatory Bowel Disease (IBD)

TLR2, TLR4 and TLR5 have been found to play a role in the pathogenesis of IBD. IBD comprises of Crohn's disease (CD) and ulcerative colitis (UC). Elevated expression of TLR4 is seen in the colonic tissue of UC and CD patients (Cairo & Podolsky, 2000), but TLR2 is found to be highly expressed in mouse manifested with colitis (Singh et al., 2005). This shows that IBD may be a consequential result of mutations and dysregulation in TLRs. Another family of PRRs, nucleotide binding oligomerization domain proteins (Nod) have been reported to contribute to IBD pathogenesis in conjunction with TLRs (Chen et al., 2007). Polymorphism in Nod2 is attributed to the development of CD.

9.4.4 Psoriasis

Psoriasis is a dermatological disorder of chronic autoimmune inflammatory nature. Expression of TLR2, TLR5 and TLR1 is altered in psoriatic individuals when compared to normal individuals (Chen et al., 2007). TLR2 is found to be highly expressed in the upper epidermis in contrast to normal skin where TLR2 is expressed in basal keratinocytes. Basal keratinocytes of the lesions also show reduced expression of TLR5 (Baker et al., 2003) and an enhanced and diffused expression of TLR1 when compared to normal skin (Curry et al., 2003). One of the mechanistic explanations of inflammatory response to psoriasis can be that the DNA released from keratinocytes in psoriatic skin binds to antimicrobial peptide cathelicidin LL37 thus mimicking bacterial DNA and triggers TLR expression on surface of immune cell/dendrocytes to activate NF-kB which controls the inflammation.

9.5 TLRs in infectious diseases

Apart from inflammatory and immune diseases associated with TLRs, TLRs are vital players in infectious diseases as well. One of these is *Mycobacterium tuberculosis* infection in which TLR2, TLR4 and TLR9 have been found to play some role. At an early stage of infection TLR2- and TLR4-knockout mice showed an increased susceptibility to the bacteria but it subsided at the later stage of infection (Tjarnlund, 2006). It has also been reported that absence of TLR2 in mice leads to aggravated inflammatory response (Drennan et al., 2004). A study reports that mice double deficient in TLR9 and TLR2 are highly susceptible to mycobacterial infection, however, single knockouts in either TLR2 or TLR9 did not show this phenomenon (Bafica et al., 2005). Another mycobacterial species, *Mycobacterium leprae,* results in various clinical manifestations associated with host immune response (Chen et al., 2007). TLR1 and TLR2 have been found to be highly expressed in patients with tuberculoid lesions whereas lepromatous lesions lack these TLRs indicating their role in the progression of tuberculoid form of the disease (Krutzik et al., 2003).

Altered expression of TLR4, TLR5 and TLR9 has been observed in *Helicobacter pylori* infection (Chen et al., 2007). TLR4 and MD-2 have been found to be expressed at significantly higher levels in gastric mucosa. This indicates the possible role of TLR4/MD-2 complex in host response to *H. pylori* derived LPS (Ishihara et al., 2004). Interestingly, TLR5 and TLR9 are located on both apical surface and basolateral surface but during *H. pylori* infection, these TLRs are not found to be expressed at the apical surface (Schmausser et al., 2005). TLR adaptor protein, MyD88, is found to be crucial in eliciting a protective host innate response against *Cryptococcus neoformans* and *Legionella pneumophila*

infections (Archer et al., 2006; Yauch et al., 2004). The response is generated via activation of TLR2. Also *Chlamydia pneumonia* infection is prevented by TLR2 and TLR4 expression (Rodriguez et al., 2006).

Lyme disease, caused by infection by spirochete *Borrelia* is also associated with TLRs. Outer surface protein A lipoprotein (OspA) is an antigen belonging to *Borrelia burgdorferi* that when docks onto TLR2 and TLR6 culminates in the induction of NFκB in human dermal endothelial cells (HMEC) (Bulut et al., 2001). TLR2/1heterodimerization is essential for the macrophages to recognize OspA and initiate desirable immune response against *B. Burgdorferi* (Chen et al., 2007). Absence of chemokine receptor XCR2 results in decreased inflammation which is considered as a novel therapeutic target for lyme disease.

Role of TLRs in viral disease progression is not yet completely elucidated, however, TLR3, TLR7, TLR8 and TLR9 have been associated with viral sensing (Kanwar et al., 2011). TLR3 and TLR9 have been conferred the foremost place in generating viral immunity. TLR3 and TLR9 recognize viral double stranded RNA and non-methylated CpG di-nucleotides (both of viral and bacterial origin) respectively. TLR7 and TLR8 too leave their signature in viral immunity by initiating IFN–α and IFN-β production in DCs and monocytes through IRAK-4 TLR adaptor (Kanwar et al., 2011). Role of TLR3 has been of prime importance in the immune response of lung epithelial cells to Influenza A virus (IAV). Influenza infection causes enhanced pulmonary expression of TLR3 in mice. However, IAV-infected TLR3-/- mice exhibited significantly reduced levels of inflammatory mediators and lower number of $CD8^+$ T lymphocytes in broncho-alveolar space (Le Goffic et al., 2006). Therefore, it can be concluded that TLR3-IAV interaction renders the body protected against debilitating host inflammatory response.

10. TLRs as adjuvant vaccines and their role in immune stimulation

Adjuvant is an agent that may stimulate the immune system and increase the response to an antigen or a vaccine without showing any antigenic property of its own. Adjuvants are used to augment the effect of a particular vaccine by putting in action the innate and then adaptive immune system in action so that the response to a vaccine is more vigorous. Adjuvants mimic molecules of bacterial or viral origin which are conserved and bear molecular signatures called PAMPs or pattern in terms of conservation. These pattern bearing molecules act as a ligand for toll like receptors (TLRs). When TLRs come in contact with their appropriate ligands, the receptor-ligand complex gives rise to innate immune response which in turn activates adaptive immune system. Since the distribution of TLRs is not limited to the innate cells such as DCs, macrophages, natural killer cells, but are also found on B cells, T cells, and other non-immune cells such as epithelial, endothelial and fibroblast, the importance of giving adjuvant based vaccines can be gauged from the fact that ligands will be able to elicit strong immune response from innate to adaptive immunity. The adjuvant simply mimics natural infection, which in turn first puts the innate then adaptive immune system on, subsequently the purpose of generation of memory cells against the desired target is achieved (Kaisho & Akira, 2002).

The role of TLRs as adjuvant receptors may be exploited to control the TLR signalling using immunity modulating reagents which may be used against the pathogens, autoimmune diseases, inflammation and cancers. Since TLRs are crucial in recognition of viral and

bacterial pathogens, current treatment aims at activating these receptors, generation of pro-inflammatory response and finally destruction of these pathogens. Ribavarin in combination with IFNα and resiquimod are currently being used as antiviral drugs. But these molecules have their own sets of limitations in terms of side effects. Agonist molecules like ANA773 and IMO-2125 have shown promising results with their respective receptors TLR7 and TLR9. TLR agonists have also been supplemented to boost immune responses to cancer vaccines. TLR7 imidazoquinoline ligand 3M-019 has been found to be a potent adjuvant for pure protein prototype vaccines (Johnston et al., 2007). TLR2 agonist SMP-105 has been approved for the bladder cancer treatment. The compound has shown strong adjuvant and antitumor activities. In experimental model, SM-105 has shown anti-tumour property. Thus TLRs as adjuvant receptors may open up new avenues of medical treatments. MPL has been approved in Europe as adjuvant vaccine. This molecule is a component of the hepatitis B vaccine and papillomavirus virus vaccine. It act as a ligand to TLR4 and activates the TRAM/TRIF pathway leading to the induction of IFNβ and regulation of CD80/86. MGN-1703 and MGN-1706 are double stem loop, non-coding DNA based adjuvants which ligate with TLR9 are being developed as anticancer agents. Another vaccine adjuvant, VAX-102, which acts as TLR5 agonist is also under trial to treat the viral infections (Elizabeth., 2010). This adjuvant vaccine if successful will provide protection from all strains of seasonal and pandemic influenza. Synthetic TLR agonists poorly reproduce the essential 'pattern' component of the larger natural ligands. To overcome this, an agonist PolyMAP, has been generated in which individual ligand is presented in a more natural linear pattern along the length of a biocompatible polymer. PolyMAP agonists can boost the immune response up to 200 times higher on a per molecule basis. PolyMAP has three key properties that contribute to the enhanced adjuvant activity and safety: (1) increased receptor avidity through cooperative, multi-valent interactions, (2) clustering of receptors through cross-linking and (3) improved solubility of TLR ligands that are otherwise difficult to use in their free form. Recently, Kasturi et al. (Kasturi et al., 2011) have reported synthetic nanoparticle adjuvant that stimulates TLR4 and TLR7, ensuing in enhanced generation of antigen-specific antibodies by synergistic action. Hence, conferring protection to lethal viral challenges in mice and inducing robust immunity against the pandemic H1N1 influenza strain in *Rhesus macaques*. Researchers developed poly(d,l-lactic-co-glycolic acid) (PGLA), a biodegradable polymer based nanoparticle to administer the TLR4 and TLR7 ligands, it was found that in comparison to the stimulation of either TLR4 or TLR7 alone, the double TLR stimulation significantly enhanced antibody response and was found to be evident even after secondary immunization.

Overall it can be concluded that adjuvants mimicking the natural molecules of viral or bacterial origin may be used to modulate the immune system resulting in the treatment of autoimmune diseases, cancers, other diseases, and when combined with vaccines, they may help in the generation of memory cells against a particular invader. The potential of adjuvant alone or as an adjuvant vaccine is yet to be fully exploited.

11. TLRs in transplantation

Transplantation is a surgical procedure by which cells, tissues or organs can be moved from one part of body to another or from one individual to another. Despite the advancement in surgical and medical sciences, the immune system remains the biggest barrier to transplantation. Till recently, the rejection of a transplant was taken as an adaptive immune

response mediated by killer T cells capable of inducing apoptosis as well as antibody secreting B cells with only small and finishing role of innate immune system like phagocytosis and complement activation. However, the emerging results have shown the importance of innate immune system in the transplant rejection via TLRs. Although direct role of TLRs are yet to be found but the activation of adaptive immune system because of TLRs may be the reason. As we know that TLRs need specific ligands to activate the signalling pathway and it is quite possible that during transplantation there is release of putative endogenous ligands such as heat shock proteins (Hsp), uric acid, hyaluronan, fibrinogen and chromatin (Goldstein, 2006). Some of these putative ligands have been seen to work with the TLRs. At the same time the role of exogenous ligands cannot be ruled out completely, which may be because of infection contracted while surgery. LPS from gram negative bacteria has been shown to activate the TLR mediated signalling pathway and create complications in graft or transplant acceptance. The recognition of alloantigen by adaptive immune system, in principle, made active because of TLRs may show increased level of complications on the development of cross reactivity with alloantigen and viral molecules. Previous studies have shown that lung, intestine and skin are more prone than kidney, heart and pancreas to acute rejection after transplantation (Wang et al., 2010). This observation may be explained that these organs which are less likely to be accepted as graft have commensals or pathogens, which in turn may activate innate immune system through TLRs leading finally to the activation of adaptive immunity. The identification of endogenous and control of exogenous TLR ligands may pave way for longer period of acceptance of transplants.

12. TLRs in trophoblast

During pregnancy the placenta is not only exposed to the maternal immune system, but also to microorganisms. It has been shown recently that in first trimester trophoblastic cells have TLR2 and TLR4 on their surface by which they can recognize and respond to invading pathogens. Interestingly, both of these TLRs show divergent response. TLR4 activation results in a more classical response, characterized by the induction of cytokine production whereas activation of TLR2 results in the induction of apoptosis. This induction of apoptosis by TLR2 may provide a mechanism by which pathogens may give rise to complicated pregnancies in the first trimester, although a clear picture remains elusive. It has been seen that in several complicated pregnancies the case of intrauterine infection was found to be the leading cause. It has been seen that during first trimester TLR2 assisted trophoblastic cell apoptosis level rises. In turn this may give rise to several medical conditions like preterm labour, IUGR, and preeclampsia. Usually, the uterine infection takes place before the implantation of trophoblast. The pathogen gets the recognition only when it is able to penetrate the placental wall and is able to reach the layer where trophoblastic cells are expressing TLRs. The TLR expression is not limited to the cell surface but has been seen also in cytoplasm may be to facilitate emergency call on infection or to face the intracellular infections, if any. The TLR4 when comes in contact with its ligand, LPS, triggers classical response whereas TLR2 induces apoptosis by coming in contact with Gram positive bacterial peptidoglycan or lipoteichoic acid, but at the same time it has been reported that recognition of these bacterial products by TLR2 requires recruitment of TLR6 or TLR1. Further studies have shown that TLR6 may show increased effect but is not essential for TLR2 mediated response and response may be executed through

TLR2 homodimer or TLR1/TLR2 heterodimer. The TLR2 induces apoptotic effect through the activation of caspases. The TLR4 interaction with LPS does not give rise to pro-apoptotic signals during first trimester because the anti-apoptotic signals generated may outweigh the pro-apoptotic signals leading to the survival of trophoblastic cells. There is also a possibility of indirect induction of apoptosis due to the high level of cytokine production such as TNF and IFN to which placenta cells are sensitive (Vikki et al., 2006).

13. Conclusion

Earlier confusion of innate immunity being nonspecific as compared to adaptive immunity got cleared with the discovery of Toll like Receptors (TLRs) which ligate with the molecules having either exogenous origin like viral or bacterial or endogenous origin like Hsp. These ligands bear molecular patterns or signatures which make them unique for their receptors. The induction by these molecules may have either positive or negative effect on the body depending on the type of ligand and TLR. It has been seen that though ligands get recognised because of molecular pattern by a specific receptor, occasionally the same receptor may ligate with two to three different molecules with each having its own signature or pattern. This may be possibly due to assistance by some co-receptors. Further study is needed to arrive at some conclusion about this enigma. The protein-ligand complex crystal structures may provide insight into the still unclear picture of TLR signalling. The TLRs have been implicated in the development of cancers, autoimmune diseases, nervous system disorders and other inflammatory diseases. TLRs agonist or antagonist may help to get the desired result. Also their role as receptor for adjuvant alone or adjuvant vaccine is being explored much vigorously than before. Role of TLRs from premature birth, complicated pregnancies to transplant rejection cannot be ignored. Moreover, their roles along with miRNA need to be probed further. Also the sources of endogenous ligands need to be discovered. Apart from receptors, the other molecules of signalling pathway too need attention to understand the process much better. The need of the time is to push the TLR research into next level where better animal models (knockout) are ready to rule out artefacts. Lastly, to design drugs, vaccines and fine tune them for lesser or ideally no side effects. Therefore, in the light of emerging information about the complexity of TLRs in immune system regulation, it can be concluded that TLRs are in fact a necessary evil. Sometimes, from their usual behaviour as a good cop by being a part of an innate immune system, a first line of defence, may turn rouge due to several compelling reasons and play bad cop. Hence, activation of TLRs is a double edged sword in therapeutics, it has the potential to mount immunity against various autoimmune, inflammatory and cancerous diseases etc. but can also promote their development and dampen immune response against them.

14. References

Akbari, O., Freeman, G.J., Meyer, E.H., Greenfield, E.A., Chang, T.T., Sharpe, A.H., Berry, G., DeKruyff, R.H. & Umetsu, D.T. (2002). Antigen-specific regulatory T cells develop via the ICOS-ICOS-ligand pathway and inhibit allergen-induced airway hyperreactivity. *Nat. Med*., Vol. 8, pp. 1024–1032.

Akiyama, H. et al. (2000) Inflammation and Alzheimer's disease. *Neurobiol Aging*, Vol.21, pp. 383-421.

Anders, H.J. (2005). A toll for lupus. *Lupus*. Vol. 14, pp. 417–422.

Arancibia, S.A., Beltran, C.J., Aguirre, I.M., Silva, P., Peralta, A.L., Malinarich, F. & Hermoso, M.A. (2007). Toll-like receptors are key participants in innate immune responses. *Biol Res.* Vol.40, pp. 97-112.

Arbour, N.C. (2000). TLR4 mutations are associated with endotoxin hyporesponsiveness in humans. *Nat Genet*, Vol.25, pp. 187–191.

Archer, K.A. & Roy, C.R. (2006) MyD88-dependent responses involving toll-like receptor 2 are important for protection and clearance of *Legionella pneumophila* in a mouse model of Legionnaires' disease. *Infect Immun,* Vol.74, pp. 3325–33.

Arroyo, S.D., Soria, A.J., Gaviglio, A.E., Rodriguez-Galan, C.M., Iribarren,P. (2011) Toll-like receptors are key players in neurodegeneration. *Inter. Immunopharmacology*, Vol.11, pp. 1415–1421.

Asea, A., Kraeft, S.K., Kurt-Jones, E.A., Stevenson, M.A., Chen, L.B. & Finberg RW et al. (2000). Hsp70 stimulates cytokine production through a CD-14-dependent pathway, demonstrating its dual role as a chaperone and cytokine. *Nat Med*, Vol.6, pp. 435–442.

Bafica, A., Scanga, C.A., Feng, C.G., Leifer, C., Cheever, A. & Sher, A. (2005). TLR9 regulates Th1 responses and cooperates with TLR2 in mediating optimal resistance to Mycobacterium tuberculosis. *J Exp Med*, Vol.202, pp. 1715–24.

Baker, B.S., Ovigne, J.M., Powles, A.V., Corcoran, S. & Fry L. (2003) Normal keratinocytes express Toll-like receptors (TLRs) 1, 2 and 5: modulation of TLR expression in chronic plaque psoriasis. *Br J Dermatol*, Vol.148, pp. 670–9.

Banchereau, J. & Steiman, R.M. (1998). Dendritic cells and the control of immunity. *Nature*, Vol.392, pp. 245-52.

Bauer, S., Hangel, D. & Yu, P. (2007). Immunobiology of toll-like receptors in allergic disease. *Immunobiology,* Vol.212, pp. 521-533.

Bell, J. K., Botos, I., Hall, P.R., Askins, J., Shiloach, J., Segal, D.M.,et al (2003).Leucine-rich repeats and pathogen recognition in Toll-like receptors. *Trends Immunol*. Vol.24, pp. 528–533.

Beutler, B. & Rietschel, E. T. (2003). Innate immune sensing and its roots: the story of endotoxin. *Nature Rev. Immunol*., Vol.3, pp. 169–176.

Beutler, B., Rehli, M. (2002). Evolution of the TIR, tolls and TLRs: functional inferences from computational biology. *Curr. Top Microbial. Immunol.* Vol.270, pp. 1-21.

Bin, L. H., Xu, L. G. & Shu, H. B. (2003).TIRP, a novel Toll/interleukin-1 receptor (TIR) domain-containing adapter protein involved in TIR signaling. *J. Biol. Chem*., Vol.278, pp. 24526–24532.

Bradley, J. R. & Pober, J. S. (2001).Tumor necrosis factor receptor associated factors (TRAFs). *Oncogene,* Vol.20, pp. 6482–6491.

Bsibsi, M.(2002) Broad expression of Toll-like receptors in the human central nervous system. *J Neuropathol Exp Neurol*, Vol.61, pp. 1013–1021.

Bulut ,Y., Faure, E., Thomas, L., Equils, O. & Arditi M. (2001). Cooperation of Toll-like receptor 2 and 6 for cellular activation by soluble tuberculosis factor and Borrelia burgdorferi outer surface protein A lipoprotein: role of Toll-interacting protein and IL-1 receptor signaling molecules in Toll-like receptor 2 signaling. *J Immunol*, Vol.167, pp. 987–94.

Burns, K., Martinon F., Esslinger C., Pahl H., Schneider P., Bodmer J.L., Marco F.D., French L. & Tschopp J. (1998). MyD88, an adapter protein involved in interleukin-1 signaling. *J. Biol. Chem*., Vol.273, pp. 12203–12209.

Cario, E. & Podolsky, D.K. (2000). Differential alteration in intestinal epithelial cell expression of toll-like receptor 3 (TLR3) and TLR4 in inflammatory bowel disease. *Infect Immun*, Vol.68, pp. 7010–7.

Carpentier PA, et al. (2005) Differential activation of astrocytes by innate and adaptive immune stimuli. *Glia*, Vol.49, pp. 360-374.

Chen, K., Huang, J., Gong, W., Iribarren P., Dunlop, N.M. & Wang, J.M. (2007). Toll like receptors in inflammation, infection and cancer. *International Immunopharmacology,* Vol.7, pp. 1271-1285.

Chen, X. & Oppenheim, J.J. (2009). Regulatory T cells, Th17 cells, and TLRs: Crucial role in Inflammation, Autoimmunity, and Cancer. *Pathways,* 888.503.3187, Issue 10.

Christensen, S.R. & Shlomchik, M.J. (2007). Regulation of lupus-related autoantibody production and clinical disease by Toll-like receptors. *Seminars in Immunology*. Vol.19, pp. 11–23.

Christensen, S.R., Kashgarian, M., Alexopoulou, L., Flavell, R.A., Akira, S. & Shlomchik, M.J. (2005) Toll-like receptor 9 controls anti-DNA autoantibody production in murine lupus. *Journal of Experimental Medicine*. Vol.202, pp. 321–331.

Curry, J.L., Qin, J.Z., Bonish, B., Carrick, R., Bacon, P. & Panella, J. et al. (2003) Innate immune-related receptors in normal and psoriatic skin. *Arch Pathol Lab Med* ,Vol.127, pp. 178–86.

Drennan, M.B., Nicolle, D., Quesniaux, V.J., Jacobs, M., Allie, N. & Mpagi, J. et al.(2004). Toll-like receptor 2-deficient mice succumb to Mycobacterium tuberculosis infection. *Am J Pathol*. Vol.164, pp. 49–57.

Elizabeth, J., Hennessy, Andrew, E., Parker, Luke A.J.O., Neill. (2010) Targeting Toll-like receptors: emerging therapeutics*, Nature Rev. Drug Discovery*, Vol. 9, pp. 293-307.

Gehring, U., Bischof, W. & Fahlbusch, B., et al. (2002). House dust endotoxin and allergic sensitization in children. *Am J Respir Crit Care Med*., Vol.166, pp. 939-944.

Gingras, S., Parganas, E., Pauw, A.D., Ihle, J.N. & Murray, P.J. (2004). Re- examination of the role of suppressor of cytokine signaling 1 (SOCS1) in the regulation of toll-like receptor signaling. *J Biol Chem*, Vol.279, pp. 54702-7.

Goldstein, D.R. (2006) Toll like receptors and acute allograft rejection, *Transplant Immuno*. Vol. 17, pp. 211–215.

Harmey, J.H., Bucana, C.D., Lu, W., Byrne, A.M., McDonnell, S. & Lynch, C. et al. (2002). Lipopolysaccharide-induced metastatic growth is associated with increased angiogenesis, vascular permeability and tumor cell invasion. *Int J Cancer* . Vol.101, pp. 415–422.

Hashimoto, C.; Hudson, K. L. & Anderson, K. V. (1988). The *Toll* gene of *Drosophila*, required for dorsal–ventral embryonic polarity, appears to encode a transmembrane protein. *Cell,* Vol.52, pp. 269–279.

Hemmi, H., Kaisho, T., Takeuchi, O., Sato, S., Sanjo, H., Hoshino, K., Horiuchi, T., Tomizawa, H., Takeda, K., Akira, S. (2002). Small anti-viral compounds activate immune cells via the TLR7 MyD88-dependent signaling pathway. *Nat. Immunol*. Vol.3, pp. 196–200.

Hoebe, K., Du, X., Georgel, P., Janssen, E.M., Tabeta, K., Kim, S.O., Goode, J., Lin, P., Mann, N., Mudd, S., Crozat, K., Sovath, S., Han, J. & Beutler, B. (2003). Identification of

Lps2 as a key transducer of MyD88-independent TIR signalling. *Nature,* Vol.424, pp. 743–748.

Horner, A.A., Van Uden, J.H., Zubeldia, J.M., Broide, D. & Raz, E. (2001). DNA-based immunotherapeutics for the treatment of allergic disease. *Immunol. Rev*. Vol.179, pp. 102–118.

Horng, T., Barton, G. M. & Medzhitov, R. (2001). TIRAP: an adapter molecule in the Toll signaling pathway. *Nature Immunol*., Vol.2, pp. 835–841.

Hsu, Y. C. Rich., Chan, H. F. Carlos, Spicer, D. J., Rousseau, C. M., Giannias, B., Rousseau, S., Ferri, E. L., (2011) *Cancer Res*, Vol. 71(5), pp. 1989-98.

Huang, Q.Q., Sobkoviak, R., Jocheck-Clark, A.R., Shi, B., Mandelin, A.M. & Tak, P.P.et al. (2009). Heat shock protein 96 is elevated in rheumatoid arthritis and activates macrophages primarily via TLR2 signaling. *J Immunol.* Vol.182, pp. 4965-73.

Ishihara, S., Rumi, M.A., Kadowaki, Y., Ortega-Cava, C.F., Yuki, T. & Yoshino N, et al. (2004). Essential role of MD-2 in TLR4-dependent signaling during Helicobacter pylori-associated gastritis. *J Immunol* ,Vol.173, pp. 1406–16.

Iwahashi, M., Yamamura, M., Aita ,T., Okamoto, A., Ueno, A. & Ogawa, N., et al. (2004). Expression of Toll-like receptor 2 on CD16+ blood monocytes and synovial tissue macrophages in rheumatoid arthritis.*Arthritis Rheum*. Vol.50, pp. 1457–67.

Jack, C.S., Arbour, N., Manusow, J., Montgrain, V., Blain, M., McCrea, E., Shapiro, A., Antel JP. (2005) TLR signaling tailors innate immune responses in human microglia and astrocytes. *JImmunol*, Vol.175, pp. 4320-4330.

Jack, C.S. et al. (2007) Th1 polarization of CD4+ T cells by Toll-like receptor 3-activated human microglia.*J Neuropathol Exp Neurol*, Vol.66, pp. 848-859.

Janeway, C. A. & Medzhitov, R. (2002). Innate immune recognition. *Annu. Rev. Immunol*., Vol.20, pp. 197–216.

Janssens, S. & Beyaert, R. (2003). Functional diversity and regulation of different interleukin-1 receptor-associated kinase (IRAK) family members. *Mol. Cell,* Vol.11, pp. 293–302.

Johnston, D., Zaidi, B., Bystryn, J.C. (2007). TLR7 imidazoquinoline ligand 3M-019 is a potent adjuvant for pure protein prototype vaccines. *Cancer Immunol Immunother,* Vol.58, Issue 8, pp.1133-41.

Kaisho, T., Akira, S., (2002) Toll-like receptors as adjuvants receptors, *Biochimica et Biophysica Acta*, Vol.1589, pp. 1-13.

Kanwar, J.R., Zhou, S.F., Gurudevan, S., Barrow, C.J., Kanwar, R.K. (2011). Toll Like Receptors Play a Role in General Immunity, Eye Infection and Inflammation: TLRs for Nanodelivery. *J Clin Cell Immunol*, Vol.2, Issue 4.

Kasturi, S.P., Skountzou, I., Albrecht, R.A., Koutsonanos, D., Hua, T., Nakaya, H.I. et. al.(2011). Programming the magnitude and persistence of antibody responses with innate immunity. *Nature*, Vol.470, pp.543-547.

Kol, A., Lichtman , A.H., Finberg, R.W., Libby, P. & Kurt-Jones EA. (2000). Cutting edge:heat shock protein (HSP) 60 activates the innate immune response:CD14 is an essential receptor for HSP60 activation of mononuclear cells. *J Immunol*, Vol.164, pp. 13–17.

Krutzik, S.R., Ochoa, M.T., Sieling, P.A., Uematsu, S., Ng, Y.W. & Legaspi A, et al.(2003) Activation and regulation of Toll-like receptors 2 and 1 in human leprosy. *Nat Med*, Vol.9, pp. 525–32.

Le Goffic, R., Balloy, V., Lagranderie, M., Alexopoulou, L., Escriou, N. & Flavell, R. et al. (2006) Detrimental contribution of the Toll-like receptor (TLR)3 to influenza Avirus-induced acute pneumonia. PLoS *Pathog* Vol.2, pp. e53.

Lebouder, E., Rey-Nores, J.E., Rushmere, N.K., Grigorov, M., Lawn, S.D., Affolter, M., Griffin ge, Ferrara, P., Schiffrin, E.J., Morgan, B.P. & Labeta , M.O. (2003) Soluble forms of Toll-like receptor (TLR)2 capable of modulating TLR2 signaling are present in human plasma and breast milk. *J Immunol*, Vol.171, pp. 6680- 9.

Lemaitre, B.; Nicolas, E., Michaut, L., Reichhart, J.M. & Hoffmann, J. A. (1996).The dorsoventral regulatory gene cassette *spatzle/Toll/cactus* controls the potent antifungal response in *Drosophila* adults. *Cell,* Vol.86, pp. 973–983.

Meylan, E., Burns, K., Hofmann, K., Blancheteau, V., Martinon, F., Kelliher, M., et al. (2004).RIP1 is an essential mediator of Toll-like receptor 3-induced NF-κB activation. *Nature Immunol*., Vol.5, pp. 503–507.

Muzio, M.; Ni, J.; Feng, P. & Dixit, V. M. (1997). IRAK (Pelle) family member IRAK-2 and MyD88 as proximal mediators of IL-1 signaling. *Science,* Vol.278, pp. 1612–1615.

Neill, L.O., (2008) Toll-like receptors in cancer, *Nature Oncogene*, Vol. 27, pp. 158–160s.

Okun, E., Griffioen, J.K., Lathia, D.J., Tang, S., Mattson, P.M., Arumugam, V.T. (2009). Toll like receptors in Neurodegenration, *Brain Res. Rev*. Vol. 59, pp. 278–292.

O'Neill, L. A., Sheedy, F. J., & Claire E. McCoy, C. E. (2011). MicroRNAs: the fine-tuners of Toll-like receptor signalling.*Nature Rev. Immunol.* Vol.11, pp. 163-175.

Parker, L.C., Prince, L.R. & Sabroe, I. (2006). Translational mini-review series on toll like receptors: networks regulated by toll like receptors mediate innate and adaptive immunity. *Clinical and Experimental Immunology.* Vol.147, pp. 199-207.

Pasare, C., Medzhitov, R. (2003). Toll pathway-dependent blockade of $CD4^+$ $CD25^+$ T cell-mediated suppression by dendritic cells. *Science*, Vol.299, pp.1033–1036.

Pessac, B. (2001) Microglia origin and development. *Bull Acad Natl Med*, Vol.185, pp. 346-33.

Poltorak, A., He, X., Smirnova, I., Liu M., Huffel C.V., Du X., Birdwell D., Alejos E., Silva M, Galanos C., Freudenberg M., Castagnoli P.R., Layton B. & Beutler B. (1998). Defective LPS signaling in C3H/HeJ and C57BL/10ScCr mice: mutations in *Tlr4* gene. *Science,* Vol.282, pp. 2085–2088.

Rodriguez, N., Wantia, N., Fend, F., Durr, S., Wagner, H. & Miethke T. (2006) Differential involvement of TLR2 and TLR4 in host survival during pulmonary infection with Chlamydia pneumoniae. *Eur J Immunol*, Vol.236, pp. 1145–55.

Savarese, E., Steinberg, C. & Pawar, R.D., et al. (2008). Requirement of Toll-like receptor 7 for pristane-induced production of autoantibodies and development of murine lupus nephritis. *Arthritis and Rheumatism*. Vol.58, pp. 1107–1115.

Schmausser, B., Andrulis, M., Endrich, S., Muller-Hermelink, H.K. & Eck M. (2005). Toll-like receptors TLR4, TLR5 and TLR9 on gastric carcinoma cells: an implication for interaction with Helicobacter pylori. *Int J Med Microbiol*, Vol.295, pp. 179–85.

Sharma, S., R. tenOever , B., Grandvaux, N., Zhou, G.P., Lin, R. & Hiscott , J. (2003). Triggering the interferon antiviral response through an IKK-related pathway. *Science,*Vol. 300, pp. 1148–1151.

Shibuya, H., Yamaguchi, K., Shirakabe, K., Tonegawa, A., Gotoh, Y., Ueno, N., Irie, K., Nishida, E. & Matsumoto, K.(1996).TAB1: an activator of the TAK1 MAPKKK in TGF-β signal transduction. *Science,* Vol.272, pp. 1179–1182.

Singh, J.C., Cruickshank, S.M., Newton, D.J., Wakenshaw, L., Graham, A. & Lan, J., et al. (2005). Toll-like receptor-mediated responses of primary intestinal epithelial cells during the development of colitis. *Am J Physiol Gastrointest Liver Physiol*, Vol.288, pp. G514–24.

Singh-Jasuja, H., Scherer, H.U., Hilf, N., Arnold-Schild, D., Rammensee, H.G. & Toes, R.E. et al. (2000). The heat shock protein gp96 induces maturation of dendritic cells and down-regulation of its receptor. *Eur J Immunol*; Vol.30, pp. 22 11–221.

Slack, J. L., Schooley, K., Timothy, P. B., Jennifer, L. M., Eva, E. Q., John E. S. & Steven K. D. (2000). Identification of two major sites in the type I interleukin-1 receptor cytoplasmic region responsible for coupling to pro-inflammatory signaling pathways. *J. Biol. Chem*., Vol. 275, pp. 4670–4678.

Sutmuller, R.P., den Brok, M.H., Kramer, M., Bennink, E.J., Toonen, L.W., Kullberg, B.J., Joosten, L.A., Akira, S., Netea, M.G. & Adema, G.J. (2006). Toll-like receptor 2 controls expansion and function of regulatory T cells. *J. Clin. Invest.* Vol.116, pp. 485–494.

Takaesu, G., Kishida, S., Hiyama, A., Yamaguchi, K., Shibuya, H., Irie, K., Tsuji, J.N. & Matsumot, K. (2000). TAB2, a novel adaptor protein, mediates activation of TAK1 MAPKKK by linking TAK1 to TRAF6 in the IL-1 signal transduction pathway. *Mol. Cell,* Vol. 5, pp. 649–658.

Takeda, K. & Akira, S. (2005). Toll like receptors in innate immunity. *The Japanese Society for Immunology*. Vol.17, pp. 1-14.

Tanaka T, et al. (2008). NF-kappaB independent signaling pathway is responsible for LPS-induced GDNF gene expression in primary rat glial cultures. *Neurosci Lett*; vol.431, pp. 262-267.

Tang SC, et al. (2007). Pivotal role for neuronal Toll-like receptors in ischemic brain injury and functional deficits. *Proc Natl Acad Sci U S A*, Vol. 104, pp. 13798-13803.

Taniguchi, T. & Takaoka, A. (2002).The interferon-α/β system in antiviral responses: a multimodal machinery of gene regulation by the IRF family of transcription factors. *Curr. Opin. Immunol*., Vol.14, pp. 111–116.

Tjarnlund, A., Guirado, E., Julian, E., Cardona, P.J. & Fernandez C. (2006). Determinant role for Toll-like receptor signalling in acute mycobacterial infection in the respiratory tract. *Microbes Infect*, Vol.8, pp. 1790–800.

Vikki, M., Abrahams, Paulomi B.A., Kim, Y.M., Shawn, L., Chavez, S., Chaiworapongsa, T., Romero, R., Mor G. (2006) *The J. Immunology*, Vol.173, pp. 4286 – 4296.

Walter ,S., et al. (2007) Role of the toll-like receptor 4 in neuroinflammation sin Alzheimer's disease. *Cell Physiol Biochem,* Vol.20, pp. 947-956.

Wang, C., Deng, L., Hong, M., Akkaraju, G.R., Inoue, J. & Chen Z.J. (Jul 2001). TAK1 is a ubiquitin-dependent kinase of MKK and IKK. *Nature,* Vol.412, pp. 346-51.

Wang, J.H., Manning, B.J., Wu, Q.D., Blankson, S., Bouchier-Hayes, D. & Redmond, H.P. (2003). Endotoxin/lipopolysaccharide activates NF-kappaB and enhances tumor cell adhesion and invasion through a beta 1 integrin-dependent mechanism. *J Immunol*. Vol.170, pp. 795–804.

Wang, S., Schmaderer, C., Kiss, E., Schmidt, C., Bonrouhi, M., Porubsky, S., Gretz, N., chaefer, L., Carsten, J., Kirschning, Zoran V., Popovic, Gröne, H.J. (2010) Recipient Toll-like receptors contribute to chronic graft dysfunction by both MyD88- and TRIF-dependent signaling, *Disease Models & Mechanisms*, Vol. 3, pp. 92-103.

Wang, Y., Chen, T., Han, C., He, D., Liu, H., An, H., Cai, Z. et. al. (2007) Lysosome-associated small Rab GTPase Rab7b negatively regulates TLR4 signaling in macrophages by promoting lysosomal degradation of TLR4. *Blood,* Vol. 110, pp. 962-971.

Wesche, H.; Henzel, W. J.; Shillinglaw, W.; Li, S. & Cao, Z. (1997). MyD88: an adapter that recruits IRAK to the IL-1 receptor complex. *Immunity,* Vol.7, pp. 837–847.

Whitmore, M.M., Iparraguirre, A., Kubelka, L., Weninger, W., Hai, T. & Williams, B.R.G. (2007). Negative Regulation of TLR-Signaling Pathways by Activating Transcription Factor-3. *J Immunol* , Vol.179, pp.3622-3630.

Wright, S.D., Ramos, R.A., Tobias, P.S., Ulevitch, R.J. & Mathison, J.C. (1990). CD14, a receptor for complexes of lipopolysaccharide (LPS) and LPS binding protein. *Science*, Vol.249, pp. 1431–1433.

Xu, Y., Tao, X., Shen, B., Horng, T., Medzhitov, R., Manley, J.L. & Tong, L. (2000). Structural basis for signal transduction by the Toll/interleukin-1 receptor domains. *Nature,* Vol.408, pp. 111–115.

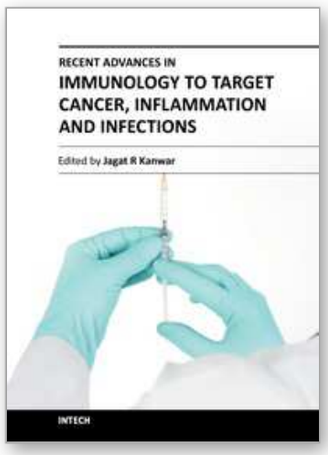
Yamaguchi, K. , Shirakabe, K., Shibuya, H., Irie, K., Oishi, I., Ueno, N., Taniguchi, T., Nishida, E. & Matsumoto K. (1995). Identification of a member of the MAPKKK family as a potential mediator of TGF-β signal transduction. *Science,* Vol. 270, pp. 2008–2011.

Yamamoto, M., Sato, S., Hemmi, H., Hoshino, K., Kaisho, T., Sanjo, H., Takeuchi, O., Sugiyama, M., Okabe, M., Takeda, K. & Akira, S. (2003). Role of adaptor TRIF in the MyD88-independent Toll-like receptor signaling pathway. *Science*, Vol.301, pp. 640–643.

Yauch, L.E., Mansour, M.K., Shoham, S., Rottman, J.B. & Levitz ,S.M. (2004).Involvement of CD14, toll-like receptors 2 and 4, and MyD88 in the host response to the fungal pathogen Cryptococcus neoformans in vivo. *Infect Immun* , Vol.72, pp. 5373–82.

Ye, H., Arron, J.R., Lamothe, B., Cirilli, M., Kobayashi, T., Shevde, N.K., Segal, D., Dzivenu, O.K., Vologodskaia, M.,Yim, M., Du, K., Singh, S., Pike, J.W., Darnay, B.G., Choi ,Y. & Wu, H. (2002). Distinct molecular mechanism for initiating TRAF6 signalling. *Nature,*Vol. 418, pp. 443–447.

Yoneyama, M., Suhara, W., Fukuhara, Y., Fukuda, M., Nishida, E. & Fujita, T. (1998). Direct triggering of the type I interferon system by virus infection: activation of a transcription factor complex containing IRF-3 and CBP/p300. *EMBO J.*, Vol.17, pp. 1087–1095.



Recent Advances in Immunology to Target Cancer, Inflammation and Infections

Edited by Dr. Jagat Kanwar

ISBN 978-953-51-0592-3
Hard cover, 520 pages
Publisher InTech
Published online 09, May, 2012
Published in print edition May, 2012

Immunology is the branch of biomedical sciences to study of the immune system physiology both in healthy and diseased states. Some aspects of autoimmunity draws our attention to the fact that it is not always associated with pathology. For instance, autoimmune reactions are highly useful in clearing off the excess, unwanted or aged tissues from the body. Also, generation of autoimmunity occurs after the exposure to the non-self antigen that is structurally similar to the self, aided by the stimulatory molecules like the cytokines. Thus, a narrow margin differentiates immunity from auto-immunity as already discussed. Hence, finding answers for how the physiologic immunity turns to pathologic autoimmunity always remains a question of intense interest. However, this margin could be cut down only if the physiology of the immune system is better understood. The individual chapters included in this book will cover all the possible aspects of immunology and pathologies associated with it. The authors have taken strenuous effort in elaborating the concepts that are lucid and will be of reader's interest.

How to reference

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Saba Tufail, Ravikant Rajpoot and Mohammad Owais (2012). Toll Like Receptors in Dual Role: Good Cop and Bad Cop, Recent Advances in Immunology to Target Cancer, Inflammation and Infections, Dr. Jagat Kanwar (Ed.), ISBN: 978-953-51-0592-3, InTech, Available from: http://www.intechopen.com/books/recent-advances-in-immunology-to-target-cancer-inflammation-and-infections/toll-like-receptors-in-dual-role-good-cop-and-bad-cop

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