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Toll-Like Receptors: Role in Inflammation and Commensal Bacteria

Abbas Ali Imani Fooladi[1], Seyed Fazlollah Mousavi[*,2], Sepideh Seghatoleslami[2], Samaneh Yazdani[3] and Mohammad Reza Nourani[3]

[1]*Microbial Products Research Center, Baqiyatallah University of Medical Sciences, Tehran, Iran*

[2]*Department of Bacteriology and Research Center of Microbiology, Pasteur Institute of Iran, Tehran, Iran*

[3]*Chemical Injury Research Center (CIRC), Baqiyatallah University of Medical Sciences, Tehran, Iran*

Abstract: TLR ligands are present on both commensal and pathogenic microbes. Intestinal epithelial cells (IECs) have been observed to be largely unresponsive to TLR ligands. This observation has partly been explained by the fact that TLR expression on IECs is sparse. The discovery of the Toll-like receptors finally identified the innate immune receptors that were responsible for many of the innate immune functions that had been studied for many years. Interestingly, TLRs seem only to be involved in the cytokine production and cellular activation in response to microbes, and do not play a significant role in the adhesion and phagocytosis of microorganisms. One member of this group, interleukin-1β (IL-1β), together with tumour-necrosis factor (TNF), is defined as an 'alarm cytokine'. It is secreted by macrophages and initiates inflammation on activation of TLRs.

INTRODUCTION

The immune response in vertebrates is broadly categorized into innate and acquired immunity [1]. The innate immune system, which was first described by Elie Metchnikoff over a century ago, is phylogenetically conserved and is present in almost all multicellular organisms [2, 3]. Innate immunity is the first line of defence against invading pathogens. The main players in innate immunity are phagocytes, such as neutrophils, macrophages and dendritic cells. These phagocytes destroy pathogens by engulfing and digesting them. They also discriminate between pathogens and self *via* the family of Toll-like receptors (TLRs) [1]. In this review we focus on characteristics of Toll-like receptors, inflammation and bacterial infection. TLRs, the first recognized family of pattern recognition receptors, can distinguish invaders through the exogenous pathogen/microb-associated molecular patterns (PAMPs/MAMPs) and tissue injury through the endogenous danger-associated molecular patterns (DAMPs) [4]. The recent discovery and characterization of the Toll-like receptor (TLR) family have incited new interest in the field of innate immunity [5]. It is already clear that these receptors have a vital role in microbial recognition, induction of antimicrobial genes and the control of adaptive immune responses. Recent evidence shows that Toll-like receptors recognize specific patterns of microbial components, especially those from pathogens, and regulates the activation of both innate and adaptive immunity [2, 6, 7].

*Address correspondence to this author at the Department of Bacteriology and Research Center of Microbiology, Pasteur Institute of Iran, Tehran, Iran; Tel/Fax: +982166405535;
E-mails: mousavi@pasteur.ac.ir, sadatff@yahoo.com

The involvement of the Toll receptors in innate immunity was first described in drosophila in 1985 by Christiane Nüsslein-Volhard [8, 9]. A year after the discovery of the role of the drosophila Toll in the host defense against fungal infection, a mammalian homologue of the drosophila Toll was identified. The TLR family is known to consist of 11 members (TLR1-TLR11), although TLR12 and13 have been recently reported in mice and no doubt more will be found in the future [10]. The TLRs are a type 1 transmembrane receptor that possesses an extracellular leucine-rich repeat domain and cytoplasmic domain homologous with that of the interleukin 1 receptor (IL-1R) family [11]. TLR family members are characterized structurally by the presence of a leucine rich repeat (LRR) domain in their extracellular domain and a toll-like/interleukin-1 receptor resistance (TIR) domain in their intracellular domain. A comparison of the amino acid sequences of the human TLRs reveals that members of the TLR family can be divided into five subfamilies: the TLR3, TLR4, TLR5, TLR2 and TLR9 subfamilies. The TLR2 subfamily is composed of TLR1, TLR2, TLR6, and TLR10; the TLR9 subfamily is composed of TLR7, TLR8, and TLR9. In the TLR2 subfamily TLR1 and TLR6 are highly similar proteins and exhibit 69.3% identity in overall amino acid sequence, but the TIR domains of both receptors are highly conserved, with over 90% identity [3, 10].

EXPRESSION OF TLR FAMILY MEMBERS

The expression of TLR family members has been elucidated in several studies. Monocytes/macrophages express mRNA for most TLRs except TLR3 [12]. Platelets also show non-self infectious danger detection molecules on their surfaces, particularly from the Toll-like receptor (TLR) family; through TLR expression, platelets can bind infectious agents and also deliver different signals for the

secretion of cytokines and chemokines [13]. Expression of TLRs in dendritic cells differs among their subsets. In humans blood dendritic cells contain two subsets, myeloid dendritic cell (MDC) and plasmacytoid dendritic cell (PDC) [14, 15]. MDCs express TLR1, 2, 4, 5, and 8, and PDCs exclusively express TLR7 and TLR9, although there are some reports that TLR7 is also expressed in MDC [16]. Immature dendritic cells mature in response to microbial components, and the expression of different TLRs shows distinct patterns during maturation [5]. Expression of TLR1, 2, 4, and 5 is observed in immature dendritic cells but decreases as the dendritic cells mature. TLR3 is expressed only in mature dendritic cells. Thus, TLRs are differentially expressed in different subsets and maturation stages of dendritic cells. Another study has examined expression of all the human TLR mRNAs in a range of tissues [17]. Mast cells have been preserved throughout evolution and have the capacity to phagocytose pathogens, process antigens, and produce inflammatory cytokines, indicating their potential role in the innate immune response against infectious organisms as well as in allergic diseases. Mast cells express TLR2, 4, 6, and 8 but not TLR5. In addition to innate immune cells, TLRs are expressed in several other types of cells that contribute to inflammatory responses [2]. The mucosal surfaces of the respiratory and intestinal tract are covered by a single layer of epithelial cells, forming a protective barrier against pathogens. In the intestine the apical surfaces of epithelial cells are continually exposed to bacteria, but this does not result in exaggerated inflammation [9, 18]. These epithelial cells elicit inflammatory responses only against pathogenic bacteria that invade into the basolateral compartment from the apical side. Thus, TLR expression is finely regulated in epithelial cells, perhaps explaining why pathogenic Gram-negative bacteria, but not commensal bacteria, induce inflammatory responses in the intestine [19]. TLR4 is not expressed on the cell surface of small intestine epithelial cells, but resides in the Golgi apparatus and is colocalized with LPS [8]. LPS is internalized and delivered to the Golgi apparatus, thereby enabling LPS-induced cell activation [20]. Therefore, the expression of TLR4 in the Golgi apparatus would be important for LPS-induced induction of chemokines by LPS in the small intestinal epithelia [8]. Renal epithelial cells are important barriers to Gram-negative pyelone phritis. Expression of TLR2 and TLR4 in renal epithelial cells is induced by IFN-γ and TNFα and contributes to the detection of bacterial invasion in the lumen of tubules and induction of the inflammatory response [6]. TLR4 is also expressed on corneal epithelial cells [21]. Microvascular endothelial cells are the first lines of defense against invading microorganisms. Human dermal endothelial cells express TLR4, indicating a possible role in detection of pathogens by endothelial cells [22].

TLR SIGNALING PATHWAYS

In vitro studies strongly suggest that TLRs have a crucial role in the recognition of microbial pathogens and that signals mediated by TLRs are crucial for mounting an effective host defense [23]. Complexes of TLR1 and TLR2 recognize lipoprotein and peptidoglycan present on the surface of Gram-positive bacteria. TLR3 is specific for double stranded RNA, an intermediate in the replication cycle of many viruses [24]. TLR4 recognizes the lipopolysaccharide surface component of Gram-negative bacteria. TLR5 reacts with flagellin, a component of bacteria flagella. TLR9 is able to distinguish between DNA sequences containing the dinucleotide CpG [2, 25]. In humans this DNA sequence is heavily methylated, while in bacteria CpG is unmethylated. TLR9 reacts only with unmethylated CpG DNA sequences and, therefore, is specific for bacteria [7]. TLRs and their ligands have been summarized in Table **1**.

Activation of signal transduction pathways by TLRs leads to the induction of various genes that function in host defence, including inflammatory cytokines, chemokines, major histocompatibility complex (MHC) and co-stimulatory molecules. Mammalian TLRs also induce multiple effector molecules such as inducible nitric oxide synthase and antimicrobial peptides, which can directly destroy microbial pathogens [3].

There are two pathways for recognition of targets by tool like receptor:

MyD88-Dependent Signaling

MyD88 was the first adaptor protein found to be critical to TLR signaling, and signaling pathways that act through this adaptor are termed 'MyD88-dependent pathways'. MyD88 can associate with all TLR types except TLR3 [16]. MyD88 is essential for the stimulation of proinflammatory cytokines such as TNF, IL-1, IL-12, or IL-6, virtually by the entire range of TLR. MyD88 contains two protein-interaction domains: an amino-terminal death domain and a carboxy-terminal TIR domain. The TIR domain of MyD88 associates with the TIR domain of TLR and the IL-1R, whereas the death domain interacts with the amino-terminal death domain of IRAK and recruits IRAK to the receptor complex (Fig. **1**) [26].

MyD88-dependent signaling *via* TLR2 and TLR4 requires the presence of TIRAP. After the adaptor protein MyD88 associates with the activated TLR, IL-1 receptor-associated kinases 1 and 4 (IRAK1 and IRAK4), and tumor necrosis factor (TNF) receptor-associated factor 6 (TRAF6) are recruited [2, 12, 16]. The IRAK1, IRAK4, TRAF6 complex then interacts with another membrane complex involving transforming growth factor-activated kinase 1 (TAK1, also known as mitogen-activated protein kinase 7 [M3K7]), TAK1-binding protein 1 (TAB1, also known as mitogen-activated protein kinase 7-interacting protein 1), and TAK1-binding proteins 2 and 3 (TAB2/3, also known as mitogen-activated protein kinase 7-interacting proteins 2 and 3) [20]. TAB1 is an activator of TAK1, whereas TAB2 functions as an adaptor linking TAK1 to TRAF6. TAK1 subsequently phosphorylates the inhibitor of NFB kinase (IKK, also known as IKBK) and mitogen-activated protein kinase 6 (MP2K6), which results in the activation of mitogen-activated protein kinases and phosphorylation of IKK. This action promotes NFB translocation to the nucleus and induces gene transcription of proinflammatory cytokines and chemokines (Fig. **1**) [12, 20].

Table 1.   Toll-Like Receptors and their Ligands

Receptor	Cell Type	Ligand	Ligand Abbreviation	Origin of Ligand
TLR1	Ubiquitous	Triacylated lipopetides	TCLDLPP	Bacteria and Mycobacteria
		Soluble factors	SF	*Neisseria meningitidis*
TLR2	DCs, PMLs, and monocytes	Heat Shock protein 70	HSP70	Host
		Peptidoglycan	PTG	Gram-positive bacteria
		Lipoprotein/lipopeptides	LP/LPPS	Various pathogens
		HCV core and nonstructural 3 protein		Hepatitis C Virus
TLR3	DC and NK cells, upregulated on epithelial and endothelial cells	Double-stranded RNA	DSRNA	Viruses
TLR4	Macrophages, PMLs, DCs, ECs, but not on lymphocytes	Lipopolysaccharides	LPS	Gram-negative bacteria
		Envelope protein	ENVP	Mouse mammary-tumor virus
		Taxol	TXL	Plants
TLR5	Monocytes, immature DCs, epithelial, NK, and T cells	Flagellin	FLGN	Bacteria
TLR6	High expression in B cells, lower on monocytes and NK cells	Zymosan	ZMS	Fungi
		Lipoteichoic acid	LTA	Gram-positive bacteria
		Diacyl lipopetides	DCLLPP	Mycoplasma
		Soluble tuberculosis factor	STF	Mycobacteria
		Outer surface protein A	OSPALP	Staphylococcus
TLR7	B cells, plasmacytoid percursor DCs	Single-stranded RNA (ssRNA)	SSRNA	Viruses
		Imidazoquinoline	IMQ	Synthetic compounds
TLR8	Monocytes, low in NK cells and T cells	Single-stranded RNA (ssRNA)	SSRNA	Viruses
		Imidazoquinoline	IMQ	Synthetic compounds
TLR9	Plasmacytoid percursor DCs, B cells, macrophages, PMLs, NK cells, and microglial cells	Unmethylated CpG-containing DNA	UMLCPGD	Bacteria, Malaria and Viruses
TLR10	B cells, plasmacytoid precursor DCs	TLR10 ligand (Not determined)	TLRL10	Not Determined
TLR11	Not Determined	Profilin-like molecule	PLP	*Toxoplasma gondii*

MyD88-Independent Signaling

MyD88-independent pathways have also been elucidated. These pathways can involve the adaptor proteins TRIF or TRAM. TLR4 and TLR3 can activate TRIF-dependent pathways without MyD88 association (Fig. **1**) [6, 10]. TRIF recruits serine threonine-protein kinase (TBK1) and mediates phosphorylation of the transcription factor interferon regulatory factor 3 (IRF3), which leads to the production of interferon and costimulatory molecules. The class-specific TLR signaling cascades enable different TLRs to trigger distinct signaling pathways and, therefore, elicit distinct actions. For example, TLR4 responses include secretion of IL-10 and IL-12, whereas TLR2 responses involve IL-8, IL-12 and IL-23. This divergence is complicated by the fact that there also seems to be cell-specific signaling. For example, endothelial cells lack TRAM, thereby restricting TLR4 signaling in these cells to the MyD88-dependent pathway [12, 16].

To sum up, diversity of TLR signaling is provided both by the TLR and the set of adaptor proteins. The correct pairing of a TLR dimer with TIR adaptors in the correct cellular location determines the outcome of TLR signaling [8].

VARIOUS TYPE OF TLRs

In each mammalian TLR seems to have a distinct function in innate immune recognition. In the past few years, dozens of TLR ligands have been identified [27, 28]. Many more ligands are yet to be identified, both for those TLRs that already have assigned ligands and those with no known ligands (Table **1**). TLR ligands are quite diverse in structure and origin. However, several common themes are emerging based on the available information [20, 29]. First, most TLR ligands are conserved microbial products (PAMPs/MAMPs) that signal the presence of infection. Second, many and perhaps all, individual TLRs can recognize several structurally unrelated ligands. Third, some TLRs require accessory proteins to recognize their ligands. Finally, although the actual mechanism of ligand recognition is still not known, available evidence indicates that mammalian
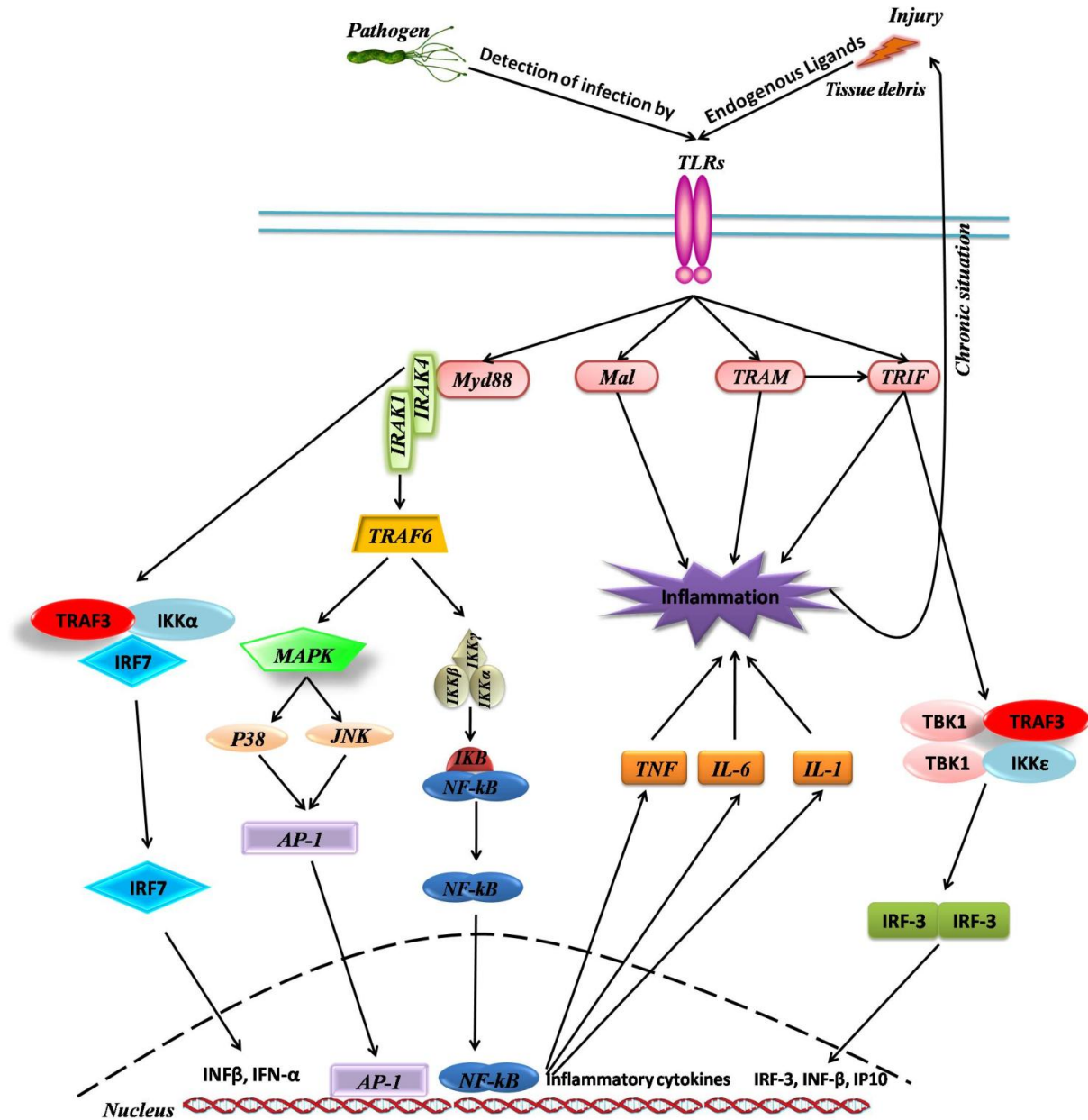
Fig. (1). Schematic representation the role of TLR signaling after infection disease and tissue injury. A variety of upstream extracellular signals activate such as TRAM, TRIF, Mal, Myd88. The Myd88 mediated pathway is shared by all the TLRs. MyD88 recruits TRAF6 and members of the IRAK family. The combination of AP-1 and NFκB controls inflammatory responses mediated by inflammatory cytokines. The MyD88 independent pathway triggers when TRIF associates with TRAF3 which binds to TBK1 and IKKε. This binding phosphorylates IRF3 that causes IRF3 dimerization and translocation into the nucleus and transcription regulation. On the other hand activation of TRAF3 leads to phosphorylation, dimerization, and nuclear localization of the transcription factors IRF7 with resultant Type I interferon (IFN) transcription.

TLRs recognize their ligands by direct binding and therefore function as PRRs [30, 31] (Table **1**).

TLR4

Human TLR4 was the first characterized mammalian Toll. It is expressed in a variety of cell types, most predominantly in the cells of the immune system, including macrophages and DCs [2]. TLR4 functions as the signal-transducing receptor for LPS. Recognition of LPS by TLR4 is complex and requires several accessory molecules. LPS is first bound to a serum protein, LBP (LPS-binding protein), which functions by transferring LPS monomers to CD14 [32]. CD14 is a high-affinity LPS receptor that can either be secreted into serum, or expressed as a glycophosphoinositol (GPI)-linked protein on the surface of macrophages. CD14-

deficient mice have a profound defect in responsiveness to LPS, showing the importance of CD14 in LPS recognition. Another component of the LPS receptor complex is MD-2 [29]. MD-2 is a small protein that lacks a transmembrane region and is expressed on the cell surface in association with the ectodomain of TLR4. Although its precise function is not known, MD-2 is required for LPS recognition by TLR4. The molecular mechanism of TLR-mediated recognition is one of the most challenging issues in Toll biology. Several lines of evidence indicate that TLR4 might, in fact, interact with LPS directly; however, this interaction is clearly aided by CD14 and MD-2 [20, 29]. In addition to LPS, TLR4 is involved in the recognition of several other ligands, including LTA53, and a heat-sensitive cell-associated factor derived from *Mycobacterium tuberculosis* [33]. TLR4 is also implicated in the recognition of the heat-shock protein HSP60 [21].

TLR2

TLR2 has been shown to be involved in the recognition of a broad range of microbial products, including: peptidoglycan from Gram-positive bacteria, bacterial lipoproteins, mycobacterial cell-wall lipoarabinomannan [34, 35] glycosyl phosphatidyl inositol lipid from *Trypanosoma Cruzi* [36], a phenol-soluble modulin produced by *Staphylococcus epidermidis*, and yeast cell walls [37]. This unusually broad range of ligands recognized by TLR2 is explained, in part, by cooperation between TLR2 and at least two other TLRs: TLR1 and TLR6. So, the formation of heterodimers between TLR2 and either TLR1 or TLR6 dictates the specificity of ligand recognition. For example, TLR2 cooperates with TLR6 for the recognition of mycoplasmal macrophage-activating lipopeptide 2 kDa (MALP-2). Interestingly, it is TLR6 that discriminates between bacterial lipoproteins, which are triacylated at the amino-terminal cysteine residue, and the diacylated mycoplasmal lipoprotein MALP-2 [38]. It is not known yet whether TLR2 heterodimerization is induced by appropriate ligands or occurs prior to ligand interaction. It is interesting to note that both TLR1 and TLR6 are expressed constitutively on many cell types, whereas expression of TLR2 is regulated and seems to be restricted to antigen-presenting cells and endothelial cells. The combinatorial recognition by TLR2 and regulation of its expression might provide an important mechanism to control cellular responsiveness to microbial products. The full repertoire of possible TLR heterodimers is not yet known, but TLR4 and TLR5, at least, are likely to function as homodimers [39]. That toxoplasmosis, cytomegalovirus, and HSV are all TLR2 ligands suggests that hat the common clinical features of TORCH diseases may relate to the common interaction between these pathogens and TLR2. Whether rubella also interacts with TLR2 is an issue that requires further investigation. The development of therapies that bind and block TLR proteins on the surface of cells might be considered in the treatment of these diseases [11].

TLR3

TLR3 has two interesting features that distinguish it from other mammalian TLRs. First, TLR3 does not contain the conserved proline residue in the position equivalent to proline of mouse TLR4. Therefore, the fact that TLR3 lacks the conserved proline at this crucial position indicated that the TLR3 signalling mechanism might differ from that of other TLRs [40]. The second interesting feature of TLR3 is that it is expressed predominantly, albeit not exclusively, in dendritic cells. Recent studies have shown that TLR3 functions as a cell-surface receptor for double-stranded RNA (dsRNA) [22]. It has been showed that TLR2, TLR3 and TLR4 pathways have been implicated in arthritis and atherosclerosis [8, 19]. Upon recognition, TLR3 induces the activation of NF-κB to increase production of type I interferons which signal other cells to increase their antiviral defenses [39].

REGULATION

Expression of TLRs is modulated by a variety of factors such as microbial invasion, microbial components, and cytokines. Infection of mice with *E. coli* induces expression of TLR2 mRNA in γδ T cells, which is thought to represent a more primitive, early line of cellular defense, preprogrammed to recognize a limited set of antigens [41]. LPS enhances expression of TLR2 in macrophages and adipocytes and causes a reduction in surface expression of the TLR4/MD-2 complex [42]. Several cytokines regulate expression of the TLRs. Colony-stimulating factor 1 can downregulate TLR9 expression in macrophages and strongly suppresses CpG DNA-induced production of inflammatory cytokines [20, 32]. Macrophage migration inhibitory factor (MIF) is an important cytokine that mediates inflammation and sepsis [10]. IFN-γ, which primes phagocytes to respond to LPS, enhances surface expression of TLR4 in human monocytes and macrophages. Expression of the *TLR2* gene in macrophages is induced by LPS and inflammatory cytokines such as IL-2, IL-15, IL-1β, IFN-γ, and TNF-α [20, 32, 42]. The pathways that transduce TLR signals in mammals have both similar and dissimilar characteristics from those in drosophila [3].

In addition to controlling the development of adaptive immunity, activation of TLRs appears to be directly involved in induction of antimicrobial activity. TLR2 activation leads to nitric oxide–dependent and –independent killing of intracellular *Mycobacterium tuberculosis* in mouse and human macrophages, respectively [34, 35, 43]. In mammals antimicrobial peptides such as defensins are produced in several kinds of epithelial cells residing in the gastrointestinal tracts, respiratory tracts, and skin [44]. Mammalian antimicrobial peptides are produced in response to microbial stimuli at the epithelial surface, the front line of defense between pathogen and host. Strong expression of TLR4 occurs in the crypts of the small intestine [45]. TLRs are likely to mediate the secretion of antimicrobial peptides, thereby regulating the direct killing of microbes at the epithelial surface. This potential involvement of TLRs in induction of mammalian antimicrobial peptides needs to be analyzed more precisely [46].

INFLAMMATION

Upon detection of PAMPs, some PRRs trigger an inflammatory response leading to efficient destruction of the

invading pathogens. Three main families of PRRs have been shown to initiate proinflammatory signaling pathways: the Toll-like receptors (TLRs), the NOD-like receptors (NLRs) and RIG-I-like receptors (RLRs) [44]. The discovery of the Toll-like receptors finally identified the innate immune receptors that were responsible for many of the innate immune functions that had been studied for many years. Interestingly, TLRs seem only to be involved in the cytokine production and cellular activation in response to microbes, and do not play a significant role in the adhesion and phagocytosis of microorganisms [47]. Recognition of PAMPs by TLRs results in the activation of different intracellular signalling cascades, which, in turn, leads to the expression of various effector molecules. One group of effector molecules consists of reactive oxygen and nitrogen intermediates, and various antimicrobial peptides, which all have direct microbicidal activity. Another group consists of the costimulatory molecules B7.1 and B7.2, which act as the second signal for T-cell activation. A third group includes adhesion molecules, chemokines and cytokines. One member of this group, interleukin-1β (L-1β), together with tumour-necrosis factor (TNF), is defined as an 'alarm cytokine' [48, 49]. It is secreted by macrophages and initiates inflammation on activation of TLRs. Subsequently, infiltrating leukocytes efficiently eliminate the infectious agents [21, 50]. Interleukin-1β causes inflammation and, more importantly, it also induces the expression of proinflammatory genes such as those that encode inducible nitric oxide synthase (iNOS), IL-6 and other cytokines or chemokines. Moreover, IL-1β increases the expression of adhesion molecules, which promote leukocyte infiltration from the blood into tissues [49].

RELATION BETWEEN COMMENSAL BACTERIA AND TLR

Despite the enormous benefits of microorganisms in host biology, relatively little is known about the molecular details of host–commensal interactions. Until recently, it was thought that the majority of benefits provided by the microflora were conferred because of the "bioactivity" of commensals [51]. Two well-known examples of this type of benefits are the roles of indigenous symbionts in energy and nutrient utilization and in resistance to infection by pathogens. In addition to these contributions to host biology, recent studies have revealed that commensals and the host are engaged in a much more intimate and complex interactions. Both *in vitro* and *in vivo* studies using human intestinal epithelial cell lines have revealed a role of nonpathogenic species of bacteria in the regulation of host cell signaling [27]. It appears that TLRs do not distinguish between pathogenic and nonpathogenic microbes on the basis of their ligand specificity. A future challenge will be to determine how the four conditions of host–microbe interactions outlined above may be fulfilled given that recognition of microbes by TLR is involved both inprotection from microbial pathogens and in mediating the benefits of colonization with symbiotic bacteria. It appears that active regulatory mechanisms, in addition to physical barriers such as anti-inflammatory cytokines, may be instrumental in setting a threshold of the immune response of host organisms to their indigenous flora. How these factors

modulate TLR signaling and allow for the induction of both TLR mediated host defense to pathogens and homeostatic interactions with the flora, while simultaneously preventing commensal-induced immunopathology, is unknown [52]. Recent studies in mice have revealed that recognition of the indigenous microflora by TLRs, best known for their role in host defense from infection, may be responsible for mediating some of the benefits of colonization by the indigenous microflora.

Commensal Bacteria of Gut and TLR

The human gut harbors an enormous quantity of commensal bacteria that have developed a symbiotic relationship with the host. The complex and dynamic ecosystem of indigenous [53]. The human colonic microbiota comprises >500 distinct bacterial species and has an important role in human nutrition and health, by promoting nutrient supply, preventing pathogen colonization and shaping and maintaining normal mucosal immunity [54, 55]. Bacterial diversity and density in the gut lumen increases from the upper to the lower gastrointestinal tract, from an almost sterile content in the stomach to 10^{12} cfu gK1 colon and faecal samples [56]. Recent studies, however, indicate that the predominant mucosa-associated community is host-specific and significantly different from lumenal and faecal bacterial communities [57]. The majority of colonic bacteria belonged to the Gram positive, anaerobic Firmicutes phylum, with the Gram negative Bacteroidetes phylum coming in second. Organisms belonging to the Proteobacteria phylum, such as *Escherichia coli*, represented a small fraction of the organisms identified in the colon [58].

Several important host factors control bacterial communities within the gut lumen and on the gut wall. For example, the protective role of secretory IgA, which promotes bacterial biofilm formation within the normal gut flora but also limits the expansion and translocation of undesirable pathogenic populations, has been long established [59]. Intestinal and colonic mucins provide additional layers of protection that influence both bacterial colonization and the host response. Other than the obvious physical and chemical constraints imposed by mucin, bacterial colonization is also controlled by novel antimicrobial activities associated with specific mucin glycans. These structures act as receptor sites, capable of promoting bacterial attachment and adherence to the gut wall [57]. Intestinal epithelial cells (IECs) are at the interface with commensal bacteria, they express pattern recognition receptors, and IEC-intrinsic innate pathways play essential roles in antimicrobial responses and immune homeostasis. In addition, distinct subsets of dendritic cells within the gut microenvironment recognize microbial-derived signals and regulate innate and adaptive immune responses. Therefore, although TLR dependent pathways are essential in normal microbial containment, the influence of IECs versus dendritic cells and other antigen presenting cells on commensal-TLR interactions in the gut remains undefined. Nevertheless, the importance of TLR-dependent pathways in bacterial compartmentalization and maintenance of mutualism supports the possibility that, compared with pathogenic microbes, commensal communities may have been an equal or greater selective evolutionary pressure to

maintain TLR-associated signaling pathways in the mammalian genome [60]. Slack and colleagues exhibited that the inability of TLR signaling–deficient mice to contain commensal bacteria in the gut lumen was independent of either non-specific defects in intestinal barrier function or impaired production and secretion of immunoglobulin A (IgA). These results highlight the essential role of TLR-dependent pathways in compartmentalization of enteric commensal bacteria [61].

A recent study by Rakoff-Nahoum and colleagues provides insight into how this happens: commensal bacteria interact with the intestinal surface and, to some degree, trigger TLR signaling. Surprisingly, this interaction is actually required to maintain the architectural integrity of the intestinal surface. Thus, it seems that the epithelium and resident immune cells do not simply tolerate commensal bacteria but are dependent on them [6]. Commensal bacteria secrete TLR ligands such as lipopolysaccharide and lipoteichoic acid, which interact in the normal intestine with a population of surface TLRs. The resultant basal signaling, which is normally ongoing, enhances the ability of the epithelial surface to withstand injury while also priming the surface for enhanced repair responses. Because many types of cells on the surface of the intestine-epithelial cells, lymphocytes between the epithelial cells, subepithelial mesenchymal cells, macrophages, and dendritic cells-express TLRs, it is not known whether this critical homeostatic mechanism is maintained by the cell populations that also respond by means of TLRs when the surface is breached. Either the disruption of TLR signaling or the removal of TLR ligands compromises the ability of the intestinal surface to withstand insult and effect repair [60, 62]. Rakoff-Nahoum *et al*. obtained further support for the validity of this hypothesis through a mirroring experiment: they found that depleting otherwise normal mice of intestinal microflora induced an equally profound injury. Reintroducing commensal bacteria into such mice, or simply adding the commensal-derived ligands for TLRs, prevented the injury. Thus, exposure of the intestinal surface to commensal-derived TLR ligands and the resulting activation of the TLR pathway are required for full health [6].

In addition to regulating the development of the intestinal and systemic immune system, recent studies suggest that alterations in the acquisition or composition of commensal bacteria may trigger or influence the course of various metabolic and inflammatory disease states [63].

For example, the development of IBD is hypothesized to be the result of dysregulated immune responses to one or more intestinal luminal antigens (loss of tolerance) in genetically predisposed individuals. While the pathophysiological features of IBD are uncontrolled, the root of the problem may lie in the defective immune tolerance to commensal bacteria and other intestinal luminal antigens [64, 65]. Experimental and clinical studies suggest that the over-expression of certain TLRs and down-regulation of TLR antagonists in intestinal epithelial cells (IEC) can be one of the underlying mechanisms leading to an improper reaction to commensal bacteria by the host. The presence of high titers of flagellin-specific antibodies in the serum of CD patients raises the possibility that flagellin from commensal

bacteria might trigger an improper immune response in the human gastrointestinal (GI) mucosa through TLR5 and that TLR5 may also play an important role in the pathogenesis of IBD. In addition, intestinal myofibroblasts express TLR2 and TLR4 and respond to LPS and LTA stimulation, and have been implicated in the development of CD-associated fibrosis [66-68].

Commensal Bacteria of Respiratory Tract and TLR

The respiratory tract is anatomically complex and constantly exposed to microorganisms in the air breathed in. The microflora of our nostrils resembles that of the skin, with colonies of commensal organisms such as micrococci, corynebacteria, staphlococci, and streptococci. *Streptococcus pyogenes* is part of the commensal flora of the nose in healthy individuals, but may cause tonsillitis and strep throat [14]. The warm, moist environment of the upper respiratory tract provides a haven for commensal bacteria including *Streptococcus, Moraxella, Neisseria,* and *Haemophplus,* species. The lower respiratory tract is generally free from microorganisms, mainly because of the efficient action of the cilia that line the tract [58]. Airway and alveolar type II epithelial cells express TLRs, and airway epithelial cells can up-regulate TLR expression to their apical surface under conditions of infection. Engagement of TLRs on these cells results in induction of antimicrobial responses. Surfactant proteins are also heavily implicated in host defence, and SP-A (surfactant protein A) can modulate signalling *via* TLRs 2 and 4 (The alveolar macrophages also provide a crucial early warning and response system for infections and, as would be expected given their roles, express functional TLRs [69]. These macrophages serve critical roles in the recognition of inhaled LPS and initiation of an inflammatory reaction. Monocyte dependent TLR-driven responses can result in the activation of airway smooth muscle and neutrophils; infiltrating neutrophils express TLRs and mediate antimicrobial signalling through their activation [53].

Cystic fibrosis (CF) lung disease is characterized by infection with *Pseudomonas aeruginosa* and a sustained accumulation of neutrophils. TLR2, TLR4, TLR5, and TLR9 expression was increased on airway neutrophils compared with circulating neutrophils in CF and bronchiectasis patients. On airway neutrophils, TLR5 was the only TLR that was significantly higher expressed in CF patients compared with bronchiectasis patients and healthy controls. Studies using confocal microscopy and flow cytometry revealed that TLR5 was stored intracellularly in neutrophils and was mobilized to the cell surface in a protein synthesis-independent manner through protein kinase C activation or after stimulation with TLR ligands and cytokines characteristic of the CF airway microenvironment. The most potent stimulator of TLR5 expression was the bacterial lipoprotein Pam3CSK4. Ab-blocking experiments revealed that the effect of Pam3CSK4 was mediated through cooperation of TLR1 and TLR2 signaling. TLR5 activation enhanced the phagocytic capacity and the respiratory burst activity of neutrophils, which was mediated, at least partially, *via* a stimulation of IL-8 production and CXCR1 signaling [59].

CONCLUSION

TLR ligands are present on both commensal and pathogenic microbes. IECs have been observed to be largely unresponsive to TLR ligands. This observation has partly been explained by the fact that TLR expression on IECs is sparse. Furthermore, cytosolic PPRs are not in direct contact with commensal microbes, and some cell membrane-bound PPRs, such as TLR5, are predominantly expressed on the basolateral surface of polarized IECs and not on the apical surface exposed to luminal antigens when the epithelium is intact [53]. Disruption of intestinal epithelial barrier integrity, for example, by inflammation or pathogen invasion, however, allows binding to basolateral TLRs and leads to an inflammatory immune response [64].

Apical and basolateral TLR9 stimulation induces distinct responses despite similar expression of TLR9 in these compartments: in contrast to basolateral TLR9 ligation, apical TLR9 signaling fails to activate NF-κB or ERK and does not lead to secretion of the proinflammatory chemokine IL-8 [70]. TLRs may not be able to distinguish between MAMPs present on pathogens and commensals as such, inflammatory responses are directed only against invading organisms penetrating the intestinal epithelial barrier. This notion is also consistent with inflammation directed toward commensal microbes observed in patients with IBD, in whom the gut barrier is compromised [53, 58]. Pathogen recognition by TLRs leads to an inflammatory immune response mediated by the NF-κB pathway, but certain enteropathogenic bacteria are able to suppress this pathway as a means to reduce host immune reaction to infection [71]. As discussed previously, nonpathogenic commensal microbes induce only transient activation of the NF-κB pathway in IECs. In addition, NF-κB plays important roles in cellular growth properties by encoding cytokines, chemokines and receptors required for neutrophil adhesion and migration, thus increasing the expression of specific cellular genes [72].

In other hand, the data alluded to previously suggesting that this hyporesponsiveness toward commensal microbes may in part be achieved by downregulation of TLR signaling through Tollip, a number of other mechanisms by which commensal microbes may inhibit NF-κB activation have recently been discovered. Given the accumulating data indicating that mucosal tolerance to commensal microbes is the result of attenuated TLR signaling and inhibition of NF-κB activation [18, 73].

REFERENCES

[1]   Akira, S.; Yamamoto, M.; Takeda, K. Role of adapters in Toll-like receptor signalling. *Biochem. Soc. Trans.,* **2003**, *31*(Pt 3), 637-642.

[2]   Reaves, T.A.; Chin, A.C.; Parkos, C.A. Neutrophil transepithelial migration: role of toll-like receptors in mucosal inflammation. *Mem. Inst. Oswaldo. Cruz.,* **2005**, *100*(Suppl 1), 191-198.

[3]   Iwasaki, A.; Medzhitov, R. Toll-like receptor control of the adaptive immune responses. *Nat. Immunol.,* **2004**, *5*(10), 987-995.

[4]   Eleftheriadis, T.; Lawson, B.R. Toll-like receptors and kidney diseases. *Inflamm. Allergy Drug Targets* **2009**, *8*(3), 191-201.

[5]   Frolova, L.; Drastich, P.; Rossmann, P.; Klimesova, K.; Tlaskalova-Hogenova, H. Expression of Toll-like receptor 2 (TLR2), TLR4, and CD14 in biopsy samples of patients with inflammatory bowel diseases: upregulated expression of TLR2 in terminal ileum of patients with ulcerative colitis. *J. Histochem. Cytochem.,* **2008**, *56*(3), 267-274.

[6]   Rakoff-Nahoum, S.; Paglino, J.; Eslami-Varzaneh, F.; Edberg, S.; Medzhitov, R. Recognition of commensal microflora by toll-like receptors is required for intestinal homeostasis. *Cell* **2004**, *118*(2), 229-241.

[7]   Witkin, S.S.; Linhares, I.M.; Giraldo, P. Bacterial flora of the female genital tract: function and immune regulation. *Best Pract. Res. Clin. Obstet. Gynaecol.,* **2007**, *21*(3), 347-354.

[8]   Lien, E.; Zipris, D. The role of Toll-like receptor pathways in the mechanism of type 1 diabetes. *Curr. Mol. Med.,* **2009**, *9* (1), 52-68.

[9]   Tarang, S.; Sodhi, A. Expression of Toll-like Receptors (TLRs) and Nucleotide-binding Oligomerization Domain (NODs) in murine peritoneal macrophages on *in vitro* treatment with Thymosin 1. *Am. J. Immunol.,* **2007**, *3*(1), 15-24.

[10] Opal, S.M.; Huber, C.E. Bench-to-bedside review: Toll-like receptors and their role in septic shock. *Crit. Care,* **2002**, *6*(2), 125-136.

[11] Kurt-Jones, E.A.; Belko, J.; Yu, C.; Newburger, P.E.; Wang, J.; Chan, M.; Knipe, D.M.; Finberg, R.W. The role of toll-like receptors in herpes simplex infection in neonates. *J. Infect. Dis.,* **2005**, *191*(5), 746-748.

[12] Krishnan, J.; Selvarajoo, K.; Tsuchiya, M.; Lee, G.; Choi, S. Toll-like receptor signal transduction. *Exp. Mol. Med.,* **2007**, *39*(4), 421-438.

[13] Garraud, O.; Cognasse, F. Platelet Toll-like receptor expression: the link between "danger" ligands and inflammation. *Inflamm. Allergy Drug Targets,* **2010**, *9*(5), 322-333.

[14] Bullens, D.M.; Ceuppens, J.L. Influence of Toll-like-receptor ligands on the dendritic cell-T cell interactions: therapeutic options for allergic diseases? Mini-review. *Inflamm. Allergy Drug Targets,* **2008**, *7*(4), 211-216.

[15] Marshak-Rothstein, A. Toll-like receptors in systemic autoimmune disease. *Nat. Rev. Immunol.,* **2006**, *6*(11), 823-835.

[16] Takeda, K.; Akira, S. TLR signaling pathways. *Semin. Immunol.,* **2004**, *16*(1), 3-9.

[17] Miggin, S.M.; O'Neill, L.A. New insights into the regulation of TLR signaling. *J. Leukoc. Biol.,* **2006**, *80*(2), 220-226.

[18] Tlaskalova-Hogenova, H.; Tuckova, L.; Mestecky, J.; Kolinska, J.; Rossmann, P.; Stepankova, R.; Kozakova, H.; Hudcovic, T.; Hrncir, T.; Frolova, L.; Kverka, M. Interaction of mucosal microbiota with the innate immune system. *Scand. J. Immunol.,* **2005**, *62*(Suppl 1), 106-113.

[19] Andrea Džumhur, T.A., Edo Džumhur, Džijan, S.; Barbić, J. Toll-like receptors and atherosclerosis. *Med. Glas.,* **2009**, *6*(1), 23-31.

[20] Hua, K.F.; Hsu, H.Y.; Chao, L.K.; Chen, S.T.; Yang, W.B.; Hsu, J.; Wong, C.H. Ganoderma lucidum polysaccharides enhance CD14 endocytosis of LPS and promote TLR4 signal transduction of cytokine expression. *J. Cell Physiol.,* **2007**, *212*(2), 537-550.

[21] Sandor, F.; Buc, M. Toll-like receptors. II. Distribution and pathways involved in TLR signalling. *Folia. Biol. (Praha),* **2005**, *51*(6), 188-197.

[22] Hari, A.; Flach, T.L.; Shi, Y.; Mydlarski, P.R. Toll-like receptors: role in dermatological disease. *Mediators Inflamm.,* **2010**, *2010*, 437246.

[23] Pilsbury, L.E.; Allen, R.L.; Vordermeier, M. Modulation of Toll-like receptor activity by leukocyte Ig-like receptors and their effects during bacterial infection. *Mediators Inflamm.* **2010**, *2010*, 536478.

[24] Alexopoulou, L.; Holt, A. C.; Medzhitov, R.; Flavell, R. A. Recognition of double-stranded RNA and activation of NF-kappaB by Toll-like receptor 3. *Nature* **2001**, *413*(6857), 732-738.

[25] Schnare, M.; Barton, G.M.; Holt, A.C.; Takeda, K.; Akira, S.; Medzhitov, R. Toll-like receptors control activation of adaptive immune responses. *Nat. Immunol.,* **2001**, *2* (10), 947-950.

[26] Netea, M.G.; van der Graaf, C.; Van der Meer, J.W.; Kullberg, B.J. Toll-like receptors and the host defense against microbial pathogens: bringing specificity to the innate-immune system. *J. Leukoc. Biol.,* **2004**, *75* (5), 749-55.

[27] Tlaskalova-Hogenova, H.; Stepankova, R.; Hudcovic, T.; Tuckova, L.; Cukrowska, B.; Lodinova-Zadnikova, R.; Kozakova, H.; Rossmann, P.; Bartova, J.; Sokol, D.; Funda, D.P.; Borovska, D.; Rehakova, Z.; Sinkora, J.; Hofman, J.; Drastich, P.; Kokesova, A. Commensal bacteria (normal microflora), mucosal immunity and chronic inflammatory and autoimmune diseases. *Immunol. Lett.,* **2004**, *93*(2-3), 97-108.

[28] Karlsson, H.; Larsson, P.; Wold, A.E.; Rudin, A., Pattern of cytokine responses to gram-positive and gram-negative commensal

bacteria is profoundly changed when monocytes differentiate into dendritic cells. *Infect. Immun.,* **2004**, *72* (5), 2671-2678.

[29]   Shin, H.J.; Lee, H.; Park, J.D.; Hyun, H.C.; Sohn, H.O.; Lee, D.W.; Kim, Y.S. Kinetics of binding of LPS to recombinant CD14, TLR4, and MD-2 proteins. *Mol. Cells,* **2007**, *24* (1), 119-124.

[30]   Ozinsky, A.; Underhill, D.M.; Fontenot, J.D.; Hajjar, A.M.; Smith, K.D.; Wilson, C.B.; Schroeder, L.; Aderem, A. The repertoire for pattern recognition of pathogens by the innate immune system is defined by cooperation between toll-like receptors. *Proc. Natl. Acad Sci. USA,* **2000**, *97*(25), 13766-13771.

[31]   Muzio, M.; Polentarutti, N.; Bosisio, D.; Manoj Kumar, P.P.; Mantovani, A., Toll-like receptor family and signalling pathway. *Biochem. Soc. Trans.,* **2000**, *28* (5), 563-566.

[32]   Lorenz, E.; Jones, M.; Wohlford-Lenane, C.; Meyer, N.; Frees, K.L.; Arbour, N.C.; Schwartz, D.A. Genes other than TLR4 are involved in the response to inhaled LPS. *Am. J. Physiol. Lung Cell Mol. Physiol.,* **2001**, *281*, L1106–L1114.

[33]   Shin, D.M.; Yang, C.S.; Lee, J.Y.; Lee, S.J.; Choi, H.H.; Lee, H.M.; Yuk, J.M.; Harding, C.V.; Jo, E. K., Mycobacterium tuberculosis lipoprotein-induced association of TLR2 with protein kinase C zeta in lipid rafts contributes to reactive oxygen species-dependent inflammatory signalling in macrophages. *Cell Microbiol.,* **2008**, *10* (9), 1893-1905.

[34]   Tjarnlund, A.; Guirado, E.; Julian, E.; Cardona, P.J.; Fernandez, C. Determinant role for Toll-like receptor signalling in acute mycobacterial infection in the respiratory tract. *Microbes Infect.,* **2006**, *8* (7), 1790-1800.

[35]   Loeuillet, C.; Martinon, F.; Perez, C.; Munoz, M.; Thome, M.; Meylan, P.R. Mycobacterium tuberculosis subverts innate immunity to evade specific effectors. *J. Immunol.,* **2006**, *177*(9), 6245-6255.

[36]   Campos, M.A.; Gazzinelli, R.T. Trypanosoma cruzi and its components as exogenous mediators of inflammation recognized through Toll-like receptors. *Mediators Inflamm.,* **2004**, *13*(3), 139-143.

[37]   Takeuchi, O.; Hoshino, K.; Kawai, T.; Sanjo, H.; Takada, H.; Ogawa, T.; Takeda, K.; Akira, S. Differential roles of TLR2 and TLR4 in recognition of gram-negative and gram-positive bacterial cell wall components. *Immunity,* **1999**, *11*(4), 443-451.

[38]   Komai-Koma, M.; Jones, L.; Ogg, G.S.; Xu, D.; Liew, F.Y. TLR2 is expressed on activated T cells as a costimulatory receptor. *PNAS,* **2004**, *101*(9), 3029-3034.

[39]   Abujamra, A.L.; Spanjaard, R.A.; Akinsheye, I.; Zhao, X.; Faller, D.V.; Ghosh, S.K. Leukemia virus long terminal repeat activates NFkappaB pathway by a TLR3-dependent mechanism. *Virology,* **2006**, *345*(2), 390-403.

[40]   Bell, J.K.; Mullen, G.E.; Leifer, C.A.; Mazzoni, A.; Davies, D.R.; Segal, D.M., Leucine-rich repeats and pathogen recognition in Toll-like receptors. *Trends Immunol.,* **2003**, *24*(10), 528-533.

[41]   Mokuno, Y.; Matsuguchi, T.; Takano, M.; Nishimura, H.; Washizu, J.; Ogawa, T.; Takeuchi, O.; Akira, S.; Nimura, Y.; Yoshikai, Y., Expression of toll-like receptor 2 on gamma delta T cells bearing invariant V gamma 6/V delta 1 induced by Escherichia coli infection in mice. *J. Immunol.,* **2000**, *165* (2), 931-940.

[42]   Lin, Y.; Lee, H.; Berg, A. H.; Lisanti, M. P.; Shapiro, L.; Scherer, P. E., The lipopolysaccharide-activated toll-like receptor (TLR)-4 induces synthesis of the closely related receptor TLR-2 in adipocytes. *J. Biol. Chem.,* **2000**, *275*(32), 24255-24263.

[43]   Rocha-Ramirez, L. M.; Estrada-Garcia, I.; Lopez-Marin, L.M.; Segura-Salinas, E.; Mendez-Aragon, P.; Van Soolingen, D.; Torres-Gonzalez, R.; Chacon-Salinas, R.; Estrada-Parra, S.; Maldonado-Bernal, C.; Lopez-Macias, C.; Isibasi, A. *Mycobacterium tuberculosis* lipids regulate cytokines, TLR-2/4 and MHC class II expression in human macrophages. *Tuberculosis (Edinb),* **2008**, *88*(3), 212-220.

[44]   Sabroe, I.; Parker, L.C.; Dower, S.K.; Whyte, M.K. The role of TLR activation in inflammation. *J. Pathol.,* **2008**, *214*(2), 126-135.

[45]   Neish, A.S. TLRS in the gut. II. Flagellin-induced inflammation and antiapoptosis. *Am. J. Physiol. Gastrointest. Liver Physiol.,* **2007**, *292*(2), G462-466.

[46]   Stanković-Đorđević, D.; Đorđević, N.; Tasić, G.; Dinić, M.; Bogdanović, M. Role of bacteria in intestinal obstruction pathophysiological processes. *Med. Biol.,* **2006**, *13*(3), 127-132.

[47]   Yoshimura, A.; Ohishi, H. M.; Aki, D.; Hanada, T. Regulation of TLR signaling and inflammation by SOCS family proteins. *J. Leukoc. Biol.,* **2004**, *75*(3), 422-427.

[48]   Tomás P. Carroll, C. M. G., Clifford C. Taggart, McElvaney, N.J. ; O'Neill, S. J. Interleukin-1, Neutrophil Elastase, and Lipopolysaccharide: Key Pro-inflammatory Stimuli Regulating Inflammation in Cystic Fibrosis. *Curr. Respir. Med. Rev.,* **2005**, *1*, 43-67.

[49]   Sabroe, I.; Whyte, M.K. Toll-like receptor (TLR)-based networks regulate neutrophilic inflammation in respiratory disease. *Biochem. Soc. Trans.,* **2007**, *35*(Pt 6), 1492-5.

[50]   Poulain-Godefroy, O.; Le Bacquer, O.; Plancq, P.; Lecoeur, C.; Pattou, F.; Fruhbeck, G.; Froguel, P. Inflammatory role of Toll-like receptors in human and murine adipose tissue. *Mediators Inflamm.,* **2010**, *2010*, 823486.

[51]   Aldridge, P.D.; Gray, M.A.; Hirst, B.H.; Khan, C.M. Who's talking to whom? Epithelial-bacterial pathogen interactions. *Mol Microbiol.,* **2005**, *55*(3), 655-663.

[52]   Rakoff-Nahoum, S.; Medzhitov, R. Role of the innate immune system and host-commensal mutualism. *Curr. Top. Microbiol. Immunol.,* **2006**, *308*, 1-18.

[53]   Rautava, S.; Walker, W. A. Commensal bacteria and epithelial cross talk in the developing intestine. *Curr. Gastroenterol. Rep.,* **2007**, *9*(5), 385-392.

[54]   Sears, C.L. A dynamic partnership: celebrating our gut flora. *Anaerobe,* **2005**, *11*(5), 247-251.

[55]   Melmed, G.; Thomas, L.S.; Lee, N.; Tesfay, S.Y.; Lukasek, K.; Michelsen, K.S.; Zhou, Y.; Hu, B.; Arditi, M.; Abreu, M. T. Human intestinal epithelial cells are broadly unresponsive to Toll-like receptor 2-dependent bacterial ligands: implications for host-microbial interactions in the gut. *J. Immunol.,* **2003**, *170*(3), 1406-1415.

[56]   Sansonetti, P. J. War and peace at mucosal surfaces. *Nat. Rev. Immunol.,* **2004**, *4*(12), 953-964.

[57]   Kelly, D.; Conway, S.; Aminov, R. Commensal gut bacteria: mechanisms of immune modulation. *Trends. Immunol.,* **2005**, *26*(6), 326-333.

[58]   Pamer, E.G. Immune responses to commensal and environmental microbes. *Nat. Immunol.,* **2007**, *8*(11), 1173-1178.

[59]   Macpherson, A.J.; Uhr, T. Induction of protective IgA by intestinal dendritic cells carrying commensal bacteria. *Science,* **2004**, *303*(5664), 1662-1665.

[60]   Hill, D.A.; Artis, D. Maintaining diplomatic relations between mammals and beneficial microbial communities. *Sci. Signal,* **2009**, *2*(98), pe77.

[61]   Haller, D. Intestinal epithelial cell signalling and host-derived negative regulators under chronic inflammation: to be or not to be activated determines the balance towards commensal bacteria. *Neurogastroenterol. Motil.,* **2006**, *18*(3), 184-199.

[62]   Qureshi, S. T.; Medzhitov, R. Toll-like receptors and their role in experimental models of microbial infection. *Genes Immun.,* **2003**, *4*(2), 87-94.

[63]   Artis, D. Epithelial-cell recognition of commensal bacteria and maintenance of immune homeostasis in the gut. *Nat. Rev. Immunol.,* **2008**, *8*(6), 411-420.

[64]   Haller, D.; Jobin, C. Interaction between resident luminal bacteria and the host: can a healthy relationship turn sour? *J. Pediatr. Gastroenterol. Nutr.,* **2004**, *38*(2), 123-136.

[65]   Furrie, E.; Macfarlane, S.; Thomson, G.; Macfarlane, G.T. Toll-like receptors-2, -3 and -4 expression patterns on human colon and their regulation by mucosal-associated bacteria. *Immunology,* **2005**, *115*(4), 565-574.

[66]   Lee, I.A.; Bae, E.A.; Hyun, Y.J.; Kim, D.H. Dextran sulfate sodium and 2,4,6-trinitrobenzene sulfonic acid induce lipid peroxidation by the proliferation of intestinal gram-negative bacteria in mice. *J. Inflamm. (Lond).,* **2010**, *7*, 7.

[67]   Harris, G.; KuoLee, R.; Chen, W. Role of Toll-like receptors in health and diseases of gastrointestinal tract. *World J. Gastroenterol.,* **2006**, *12*(14), 2149-2160.

[68]   Yan, F.; Polk, D. B., Commensal bacteria in the gut: learning who our friends are. *Curr. Opin. Gastroenterol.,* **2004**, *20*(6), 565-571.

[69]   Chaudhuri, N.; Dower, S.K.; Whyte, M.K.; Sabroe, I. Toll-like receptors and chronic lung disease. *Clin. Sci. (Lond),* **2005**, *109*(2), 125-133.

[70]   Duramad, O.; Fearon, K.L.; Chang, B.; Chan, J.H.; Gregorio, J.; Coffman, R.L.; Barrat, F.J. Inhibitors of TLR-9 act on multiple cell subsets in mouse and man *in vitro* and prevent death *in vivo* from systemic inflammation. *J. Immunol.,* **2005**, *174*(9), 5193-5200.

[71] Heimesaat, M.M.; Fischer, A.; Siegmund, B.; Kupz, A.; Niebergall, J.; Fuchs, D.; Jahn, H.K.; Freudenberg, M.; Loddenkemper, C.; Batra, A.; Lehr, H.A.; Liesenfeld, O.; Blaut, M.; Gobel, U.B.; Schumann, R.R.; Bereswill, S. Shift towards pro-inflammatory intestinal bacteria aggravates acute murine colitis *via* Toll-like receptors 2 and 4. *PLoS One,* **2007**, *2*(7), e662.

[72] Imanifooladi, A. A.; Yazdani, S.; Nourani, M.R. The role of nuclear factor-kappaB in inflammatory lung disease. *Inflamm. Allergy Drug Targets,* **2010**, *9*(3), 197-205.

[73] Janssens, S.; Beyaert, R. Role of Toll-like receptors in pathogen recognition. *Clin. Microbiol. Rev.,* **2003**, *16*(4), 637-646.