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REVIEW

Toll-Like Receptors' Pathway Disturbances are Associated with Increased Susceptibility to Infections in Humans

Josias Brito Frazão · Paolo Ruggero Errante · Antonio Condino-Neto

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Abstract Toll-like receptors (TLRs) sense microbial products and play an important role in innate immunity. Currently, 11 members of TLRs have been identified in humans, with important function in host defense in early steps of the inflammatory response. TLRs are present in the plasma membrane (TLR1, TLR2, TLR4, TLR5, TLR6) and endosome (TLR3, TLR7, TLR8, TLR9) of leukocytes. TLRs and IL-1R are a family of receptors related to the innate immune response that contain an intracellular domain known as the Toll-IL-1R (TIR) domain that recruits the TIR-containing cytosolic adapters MyD88, TRIF, TIRAP and TRAM. The classical pathway results in the activation of both nuclear factor κB and MAPKs via the IRAK complex, with two active kinases (IRAK-1 and IRAK-4) and two non-catalytic subunits (IRAK-2 and IRAK-3/M). The classical pro-inflammatory TLR signaling pathway leads to the synthesis of inflammatory cytokines and chemokines, such as IL-1β, IL-6, IL-8, IL-12 and TNF-α. In humans, genetic defects have been identified that impair signaling of the TLR pathway and this may result in recurrent pyogenic infections, as well as virus and fungi infections. In this review, we discuss the main mechanisms of microbial recognition and the defects involving TLRs.

Keywords Toll-like receptors · Immunodeficiencies · Recurrent infections · NF-kappaB · IRAK-4

Abbreviations

AD	Autosomal dominant
APCs	Antigen-presenting cells
BCG	Bacillus Calmette-Guérin
BTK	Brutons's tyrosine kinase
CD62L	CD62 ligand
CNS	Central nervous system
CVID	Common variable immunodeficiency
DCs	Dendritic cells
dsRNA	Double-stranded RNA
EDA-ID	Ectodermal dysplasia with immunodeficiency
HClO	Hypochlorous acid
HO-1	Heme oxygenase 1
HSE	Herpes simplex virus-encephalitis
HSV-1	Herpes simplex virus type 1
IFN-γ	Interferon-gamma
IKK	Inhibitor of NF-κB kinase complex
IL-8	Interleukin-8
IRAK-4	IL-1 receptor-associated kinase 4
LPS	Lipopolysaccharide
LTA	Lipoteichoic acid
MALP-2	Macrophage-activating lipopeptide-2
MAPKs	Mitogen-activated protein kinases
MBL	Mannose-binding lectin
MyD88	Myeloid differentiation factor 88
NADPH	Nicotinamide adenine dinucleotide oxidase
NEMO	NF-κB essential modulator
NF-κB	Nuclear factor kappaB
NLRPs	NOD leucine-rich repeat and pyrin domain containing proteins
NLRs	Leucine-rich repeat-containing receptors
PAMPs	Pathogen-associated molecular patterns
PBMCs	Peripheral blood mononuclear cells
PDC	Plasmacytoid DCs
PIC	Poly-riboinosinicribocytidylic acid
PID	Primary immunodeficiency diseases
PMN	Polymorphonuclear cells

J. B. Frazão (✉) · P. R. Errante · A. Condino-Neto
Department of Immunology, Institute of Biomedical Sciences, University of São Paulo, 1730, Lineu Prestes Avenue, São Paulo, SP 05508-000, Brazil
e-mail: britoj@usp.br

PRRs	Pattern-recognition receptors
RIG-I	Retinoic acid-inducible gene I protein
ssRNA	Single-stranded RNA
TIR	Toll-IL-1R domain
TIRAP	TIR domain-containing adapter protein
TLRs	Toll-like receptors
TRAM	TRIF-related adapter molecule
TRIF	TIR domain-containing adapter-inducing IFN-β
Xid	X-linked immune deficiency
XLA	X-linked agammaglobulinemia

Introduction

Since birth, the newborn is constantly exposed to several types of antigens in the form of microbial agents and to inert agents that are inhaled from the atmosphere or ingested during alimentation. The immune system function is to prevent or delay the local establishment, systemic access and wide dissemination of these agents (Murray and Wynn 2011). An adequate immune response is a guarantee of the integrity and resistance of the organism to infections. This integrity is maintained by the synergic activity of specific and non-specific immunity that guarantees the resistance to infections, promotes surveillance against tumor, rejects non-compatible transplant, and also prevents autoimmune diseases (Costa-Carvalho et al. 1998). The reaction of the host against infections is a complex event, in which many genetic variations of the immune system can influence the ability to respond in an effective manner to challenges represented by microorganisms (WHO 1997). Some characteristics of the host, such as age, nutritional state, and social economic status, have to be considered due to its nonspecific influence in the defense against infectious agents (Zemskov et al. 2005). Immune response can be exerted by unspecific mechanisms called innate immunity or natural immunity (Galli et al. 2011). The establishment of a microorganism on the tissue is blocked by natural barriers that are physical, chemical barriers represented by the skin and mucosa, and antimicrobial substances produced locally (Ryser et al. 1988). Any change in the structure or the integrity of these natural barriers can favor the establishment of microorganisms (Shearer et al. 1994). Natural or innate immunity is the first line of defense against microorganisms and has no specificity. As a consequence, there is no immunologic memory and its responsiveness depends predominantly on phagocytes (neutrophils and macrophages) and natural killer cells that are responsible for the elimination of virus-infected cells and some tumoral cells (Chapel et al. 2003). Neutrophils are produced in the bone marrow and present a life span of 6.5 h in the peripheral blood (Shearer et al. 1994). These are present in high amount in the blood and absent in the tissue, but undergo a process called chemotaxis, which allows them to migrate toward sites of infection or inflammation. Cell surface receptors allow neutrophils to detect chemical gradients of molecules such as interleukin (IL)-8, interferon (IFN)-γ, C3a, C5a and leukotriene B4 directing these cells through migration. Neutrophils are highly motile and quickly congregate at a focus of infection, attracted by cytokines expressed by activated endothelium, mast cells, and macrophages. Neutrophils express and release cytokines, which in turn amplify inflammatory reactions by several other cell types (Ear and McDonald 2008). In addition to recruiting and activating other cells of the immune system, neutrophils play a key role in the front-line defense against invading pathogens. Neutrophils have three strategies for directly attacking microorganisms: phagocytosis (ingestion), release of soluble anti-microbials (including granule proteins) and generation of neutrophil extracellular traps (Hickey and Kubes 2009). Macrophages are cells produced by the differentiation of blood monocytes in tissues (Krombach et al. 1997); their function is to engulf and digest cellular debris and pathogens, either as stationary or as mobile cells. They also stimulate lymphocytes and other immune cells to respond to pathogens. They are specialized phagocytic cells that respond to foreign substances, infectious microbes, and cancer cells through destruction and ingestion. Macrophages can be identified by specific expression of a number of proteins including CD14, CD11b, F4/80(mice)/EMR1(human), lysozyme M, MAC-1/MAC-3, and CD68 by flow cytometry or immunohistochemical staining (Khazen et al. 2005). Both cell types, neutrophils and macrophages during phagocytosis, present "respiratory burst" activity which involves the activation of the NADPH-oxidase enzyme, producing large quantities of superoxide, a reactive oxygen species with microbicidal activity. The superoxide dismutates spontaneously or through catalysis via enzymes known as superoxide dismutases (Cu/ZnSOD and MnSOD) to hydrogen peroxide, which is then converted to hypochlorous acid (HClO), by the green heme enzyme myeloperoxidase. It is thought that the bactericidal properties of HClO are enough to kill bacteria phagocytosed by the neutrophil, but this may instead be a step necessary for the activation of proteases (Segal 2005).

Recognition of Pathogenic Microorganisms

During infections, cells of the innate immune system recognize conserved structures of the microbe called pathogen-associated molecular patterns (PAMPs) (Akira

et al. 2006). Complement factor 1q, C-reactive protein, and fibronectin also recognize microbial structures, but are not usually considered pathogen-recognition receptors (Botto et al. 2009). Cells of the innate immune system recognize PAMPs by means of germline-encoded pattern-recognition receptors, which allow for semi-specific recognition of pathogens. An example of such a receptor is the macrophage mannose receptor (Ezekowitz et al. 1990). In 1992, Charles Janeway Jr. and Ruslan Medzhitov took the field of innate immunity in a new direction with his concept of selective recognition of conserved microbial structures by pattern-recognition receptors (PRR) (Janeway 1992). There are four classes of PRR: Toll-like receptors (TLRs), C-type lectin receptors, nucleotide-binding oligomerization domain leucine-rich repeat-containing receptors (NLRs), and retinoic acid-inducible gene I protein (RIG-I) helicase receptors (Akira et al. 2006). These are constituents of microbial cell wall components, microbial nucleic acids, or metabolic products. We use the term PRR to mean a cellular receptor that causes activation of innate immunity; we do not include molecules that microorganisms use for attachment to and invasion of host cells.

Toll-Like Receptors

Toll-like receptors are one of the four families of PRR that was first discovered in 1985; they received their name from their similarity to the protein coded by the Toll gene identified in *Drosophila melanogaster* by Christiane Nüsslein-Volhard (Hansson and Edfeldt 2005). The role of TLRs in antimicrobial defense was described in 1996 by Bruno Lemaitre and colleagues (Lemaitre et al. 1996), when it was observed that fruit flies lacking the insect homolog of a toll receptor rapidly died from aspergillosis. This phenomenon was explained by the fact that all the extracellular domains of these receptors contain leucine-rich repeats and a cytoplasmic Toll/IL-1R (TIR) domain, which is crucial for TLR downstream signaling (O'Neill and Dinarello 2000), turning it into a critical component of the innate immune system and helping to protect hosts from infectious disease through the recognition of PAMPs (Rock et al. 1998). The PAMPs recognized by TLRs are diverse, but include molecules expressed by bacteria, fungi, viruses, and protozoa. Since then, mammalian TLRs have been discovered in mice and humans and are reported to be expressed in both myeloid and lymphocytic lineages (Akira and Takeda 2004). TLRs sense microbial products and play an important role in innate immunity. Currently, 11 members of TLRs have been identified in humans, with important functions in innate immunity host defense and early steps of the inflammatory response. TLRs are present in the plasma membrane (TLR1, TLR2, TLR4, TLR5, TLR6) and endosome (TLR3, TLR7, TLR8, TLR9) of leukocytes. TLRs and IL-1R family are receptors related to the innate immunity that contain an intracellular domain known as the TIR domain that recruit the TIR-containing cytosolic adapters myeloid differentiation factor 88 (MyD88), TIR domain-containing adapter-inducing IFN-β (TRIF), TIR domain-containing adapter protein (TIRAP), and TRIF-related adapter molecule (TRAM). The classical pathway results in the activation of both nuclear factor (NF)-κB and mitogen-activated protein kinases (MAPKs) via the IRAK complex, which has two active kinases (IRAK-1 and IRAK-4) and two noncatalytic subunits (IRAK-2 and IRAK-3/M). Once activated by a ligand, TLRs trigger an intracellular kinase cascade of signaling by intermediate adapter molecules, culminating in the activation of the inhibitor of the NF-κB (IκB) kinase (IKK) complex (Doyle and O'Neill 2006). The regulation of NF-κB following stimulation with lipopolysaccharide (LPS) occurs via activation of two pathways. In the classical pathway activation, NF-κB is a transcription factor in the cytoplasm of resting cells through association with NF-κB inhibitor (IκB) proteins. After cell stimulation, IκB is phosphorylated at two conserved critical amino-terminal serine residues by the IKK complex, leading to their ubiquitination and subsequent degradation. The IKK complex is composed of two catalytic subunits, IKKα and IKKβ, and IKKγ/NEMO (NF-κB essential modulator) (Orange et al. 2005). The degradation of IκB results in the translocation of NF-κB dimers to the nucleus, where they bind to specific DNA sites with regulation of gene transcription. This phenomenon leads to the synthesis of inflammatory cytokines and chemokines, important to host defense in early steps of the inflammatory response (Schmitz et al. 2001) (Fig. 1). All TLRs have the capacity to initiate inflammation through their ability to induce the production of an important NF-κB target, inducing NF-κB binding to discrete nucleotide sequences in the upstream regions of genes, causing its mRNA expression and production of pro-inflammatory cytokines such as tumor necrosis factor (TNF)-α (Akira and Takeda 2004; Akira et al. 2006; Beutler 2004) and IL-6, thereby regulating their expression (Baeuerle and Henkel 1994). Although TLRs have the capacity to induce divergent signaling pathways resulting in the production of additional NF-κB-dependent as well as NF-κB-independent cytokines, the production of TNF-α provides a measure of the integrity of TLRs itself and of the NF-κB-dependent pathway. In addition to their role in innate immunity, TLRs and other PRR activate antigen-presenting cells (APCs) and bridge innate and adaptive immunity by coordinating responses of T cells and B cells (Iwasaki and Medzhitov 2010; Netea et al. 2004). TLRs bind and become activated by different ligands, which in turn are located on different type of organisms and structures, present different adapters to respond to

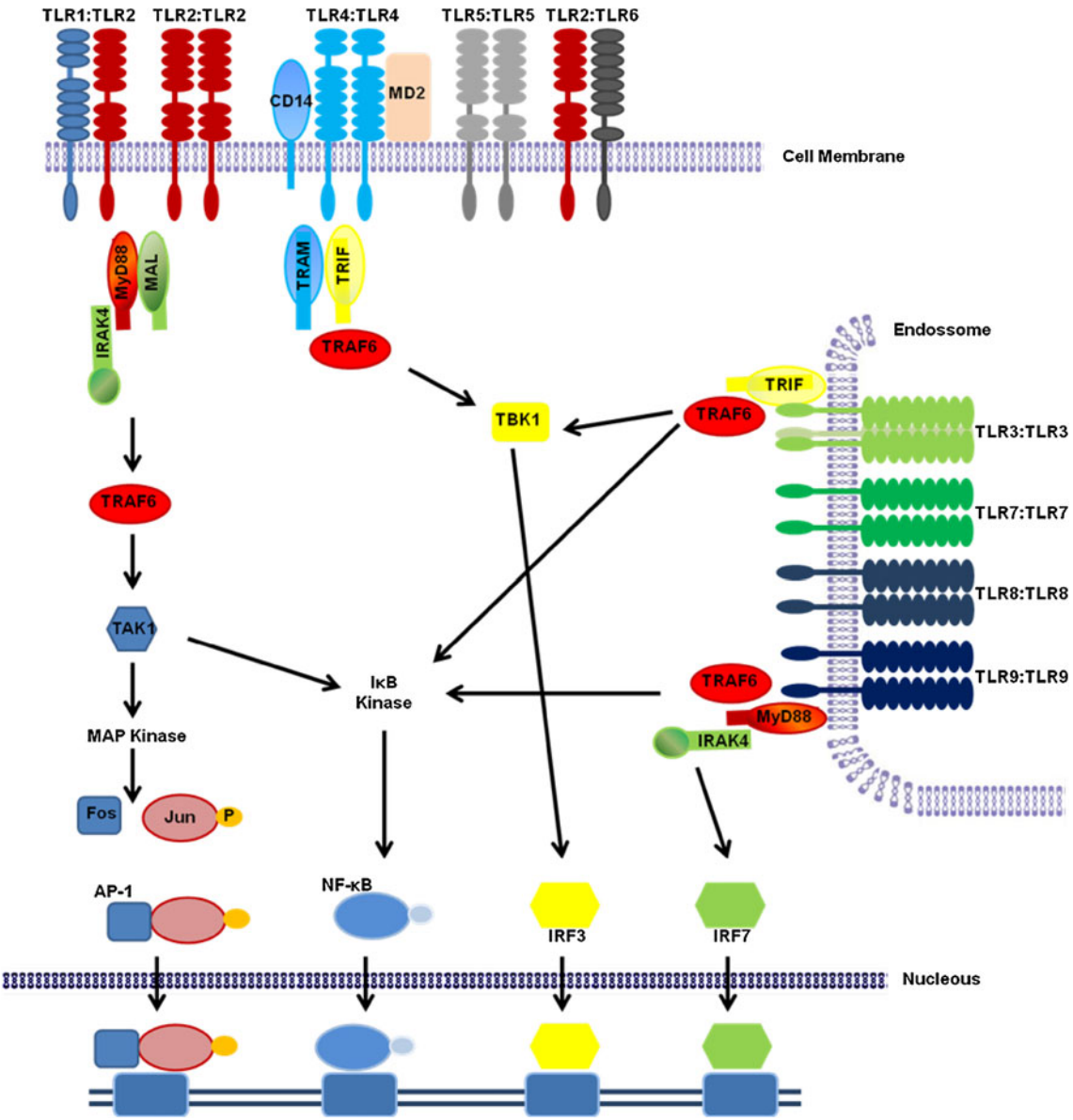

Fig. 1 Signaling pathway of Toll-like receptors. TLRs function in dimers; though most TLRs appear to function as homodimers, TLR2 forms heterodimers with TLR1 or TLR6. TLR4 recognition of LPS requires MD2 and CD14. TLR signaling is divided into two distinct signaling pathways, the MyD88-dependent and TRIF-dependent pathway. The MyD88-dependent response occurs on dimerization of the TLR receptor, and is utilized by every TLR except TLR3. Its primary effect is the activation of NF-κB. Ligand binding that occurs in the receptor recruits the adapter protein MyD88, recruits IRAK4, and phosphorylates and activates the protein TRAF6, which in turn polyubiquitinates the protein TAK1 and phosphorylates IKKβ. This then phosphorylates IκB causing its degradation and allows NF-κB to diffuse into the cell nucleus and activate transcription of inflammatory cytokines and adhesion molecules. TRL3 uses the TRIF-dependent pathway, and dsRNA leads to activation of the receptor, recruiting the adapter TRIF. TRIF activates the kinases TBK1, signaling the complex to phosphorylates IRF3 allowing its translocation into the nucleus and production of IFN-type I

activation, and are located at the cell surface and internal cell compartment, as summarized in Table 1. The significance of individual TLRs in protection against infectious disease has been clearly demonstrated in murine models and is slowly being demonstrated in primary immunodeficiency patients as they are being recognized and reported in the literature (Picard et al. 2011).

Defects in TLR Pathways Related to Immunodeficiencies in Humans

In primary immunodeficiency diseases (PID) affecting PRR, susceptibility to infections is increased, and in study models involving specific TLRs that are genetically removed or altered in mice, result in increased susceptibility

Table 1 TLRs, ligands, adapters, location, and cell type involved

Receptor	Recognize	Ligand location	Adapter	Location	Cell type
TLR1	Triacyl lipopeptides	Bacteria	MyD88	Cell surface	PMN, monocyte, macrophage, B cell, DC cell
TLR2	Glycolipids, LTA, HSP70, zymosan	Bacteria Gram-positive, cell host	MyD88	Cell surface	Monocyte, myeloid DC, mast cell
TLR3	Double-stranded DNA	Virus	TRIF	Endosome	DC, B cell
TLR4	LPS, HSP, fibrinogen, heparan sulfate fragments, hyaluronic acid fragments	Bacteria Gram-negative	MyD88/ TRIF/ TRAM	Cell surface	Monocyte, macrophage, myeloid DC, mast cell, intestinal epithelium
TLR5	Flagellin	Bacteria	MyD88	Cell surface	Monocyte, macrophage, DC, intestinal epithelium
TLR6	Multiple diacyl lipopeptides	Mycoplasma	MyD88	Cell surface	Monocyte, Macrophage, Mast cell, B cell
TLR7	Imidazoquinoline, loxoribine (guanosine analog), bropirimine, single-stranded RNA	Small synthetic components	MyD88	Endosome	Monocyte, macrophage, plasmocytoid DC, B cell
TLR8	Small synthetic compounds, single-stranded RNA		MyD88	Endosome	Monocyte, macrophage, DC, mast cell
TLR9	Unmethylated CpG-DNA	Bacteria	MyD88	Endosome	Monocyte, macrophage, plasmocytoid DC, B cell
TLR10	Unknown	Unknown	Unknown	Cell surface	Monocyte, macrophage, B cell
TLR11	Profilin	*Toxoplasma gondii*, uropathogenic *Escherichia coli*	MyD88	Cell surface	Monocyte, macrophage, liver cells, kidney cells, bladder epithelium

HSP heat shock protein

Table 2 Defects in innate immunity associated with TLRs and signaling

Disease	Functional defect	Associated features
Borreliosis/Lyme disease	TLR1/2(heterodimer) 4, 6	TLR upregulation with eventual exacerbation of disease
Candida albicans	TLR2, 4-CD14, 6	TLR function and signaling lead to disease progression
Herpes simplex/*Varicella zoster*	TLR2, 3, 9	Select TLR-2 polymorphism associated with disease severity TLR-3 and help in viral clearance
Leprosy	TLR1, 2	TLR function and signaling lead to disease progression
Staphylococcus aureus	TLR2, 6	TLR-2 polymorphism associated with disease severity TLR-2, -6 function and signaling lead to disease progression
Syphilis	TLR2, 4/5 (heterodimer)	TLR activated the immune system
Legionella pneumophila	TLR2, 5	TLR-5 polymorphism associated with disease severity
Vaccinia	TLR2, 3, 4	TLR helps in viral clearance, but is targeted by viral products which suppress host defense
Verruca/molluscum contagiosum	TLR3, 7, 9	TLR-3, 9 help in immune activation TLR-7, possible association with disease exacerbation
Yersinia spp.	TLR2, 4-CD14	Pathogen exploits TLR pathway to survive
Herpes virus encephalitis	TLR3-dependent IFN-α, -β, -λ induction	Herpes simplex virus 1 encephalitis and meningitis
IRAK-4 deficiency	TLR and IL-1 receptor-IRAK signaling pathway	Bacterial infections (pyogenic)
EDA-ID	NF-κB signaling pathway	Various infections (mycobacteria and pyogenic)

to pathogens expressing PAMPs recognized by the given TLRs (Kopp and Medzhitov 2003). The best characterized TLR is TLR4, which recognizes the Gram-negative product LPS, fungal mannan, parasitic phospholipids, viral envelop proteins, and host heat shock proteins. Another TLR with antibacterial proprieties is TLR2 that in combination with

TLR1 recognizes triacylated lipopeptides, or in combination with TLR6 recognizes diacylated lipopeptides, bacterial lipoteichoic acid (LTA) and TLR5, which recognizes bacterial flagellin. The antiviral TLRs are: TLR3, which recognizes double-stranded RNA; TLR7 and TLR8, which recognize single-stranded RNA (ssRNA); and TLR9 which recognizes unmethylated CpG motifs that exist in both viral and bacterial DNA (Akira and Hemmi 2003; O'Neill and Bowie 2007). The innate immune recognition of Gram-positive bacteria strongly depends on TLR2 (Hoebe et al. 2005; Takeuchi et al. 2000) which is expressed on the cell surface and within endosomes of APCs such as monocytes, macrophages, and dendritic cells (DCs) (Hornung et al. 2002). It recognizes various staphylococcal cell wall components including LTA (Seo et al. 2008), lipoproteins (Buwitt-Beckmann et al. 2005), and peptidoglycan (Dziarski and Gupta 2006), albeit recognition of the latter is controversially discussed (Dziarski and Gupta 2005; Travassos et al. 2004). This broad range of different bacterial structures described as TLR2 ligands can be explained by heterodimer formation between TLR2 and other TLRs, such as TLR1 or TLR6 (Jin et al. 2007; Kang et al. 2009). The effects of *Mycobacterium bovis* bacillus Calmette-Guérin (BCG) recognition were evaluated by viable BCG with decrease of TLR2, *CYBB* expression and superoxide release by human peripheral blood mononuclear cells (PBMCs). Heat-killed BCG or viable BCG increases TNF-α release by PBMC, suggesting potential use in patients with PID (Moreira et al. 2012). The most common infections associated with TLR and signaling defects are summarized in Table 2. However, not only PID increases the susceptibility to infections. Different versions of certain DNA sequence in a given locus are called alleles and the coexistence of multiple alleles at a *locus* is called polymorphism. Polymorphisms present in the population in genes encoding PPRs may also be an important factor that may also contribute to susceptibility to infection.

TLR2, in Combination with TLR1, Recognizes Triacylated Lipopeptides

TLR1 with TLR2 recognizes bacterial lipopeptides triacil, and TLR2 recognizes peptidoglycan, lipoprotein, LTA, bacterial porins, and viral hemagglutinin. The role of TLR with TLR1 has been observed in human leprosy (Krutzik et al. 2003), and the expression of TLR2 was observed in human Schwann cells associated with a mechanism of nerve damage in leprosy (Oliveira et al. 2003). While TLR2 is the main recognition receptor for *Borrelia* spp., there is a dominant role for TLR1/TLR2 heterodimers in the induction of the early inflammatory response by *Borrelia* spirochetes in humans. After infection with *Borrelia* species, the risk for developing Lyme disease is significantly variable among individuals, supported at the moment in part by polymorphism of TLR1/TLR2 (Oosting et al. 2011). Polymorphisms in TLR2 were associated with increased viral shedding and lesional rate in patients with genital herpes simplex virus type 2 (HSV-2) infection (Bochud et al. 2007), and TLR2 in association with TLR4-CD14 was associated with suppression of immune response against Yersina V-antigen (Sing et al. 2002) or block of NF-κB signaling in macrophages by protein P of *Yersinia enterolocolitica* (Ruckdeschel et al. 2001).

TLR2, in Combination with TLR6, Recognizes Diacylated Lipopeptides and Bacterial LTA of Mycoplasmal Compounds

Beside TLR2, the mannose-binding lectin (MBL) and CD36, both able to directly interact with LTA, were found to have an important role during the host immune response against *Staphylococcus aureus* (Hoebe et al. 2005; Ip et al. 2008; Jimenez-Dalmaroni et al. 2009). Whereas membrane-bound CD36 was found to augment TLR2/6-dependent recognition of *S. aureus* and its LTA via improving their intracellular uptake (Stuart et al. 2005), soluble CD36 and MBL were described as enhancing LTA-mediated activation by improving LTA delivery to the TLR2/6 heterodimer (Ip et al. 2008; Jimenez-Dalmaroni et al. 2009). Despite strong evidence of a major role for LTA in the immune activation by Gram-positive bacteria (Deininger et al. 2003; Morath et al. 2001, 2002), recent reports suggest that not LTA, but lipoproteins are dominant immunobiologically active structures of *S. aureus* (Hashimoto et al. 2006b, 2007) and that contaminating lipoproteins in the LTA preparations are responsible for LTA-mediated immune activation (Hashimoto et al. 2006a, 2006b, 2007). Contrary to the latter investigators (von Aulock et al. 2007), Sebastian Bunk (Bunk et al. 2010) found that LTA of a mutant *S. aureus* strain lacking lipoproteins (Δlgt-LTA) and wild-type LTA were equipotent in inducing cytokine release from human whole blood and described that cytokine induction by Δlgt-LTA in human peripheral blood was critically dependent on surface immobilization of LTA. This provides evidence that recognition of Δlgt-LTA requires the coexpression of TLR2, TLR6, and CD14. Another group, assessing the role of TLR2 and CD47 costimulation in regulating polymorphonuclear (PMN) transmigration, demonstrated that treatment with macrophage-activating lipopeptide-2 (MALP-2, a TLR2/6 agonist) or anti-human CD47 monoclonal antibodies delayed human PMN transfilter migration, while combined treatment led to further delayed inhibition, and stimulation of PMNs with MALP-2 resulted in an increase in surface expression of CD11b, but not CD47 (Chin et al.

2009). TLR2 and CD47 exhibit cross-talk mechanisms through an MyD88-dependent manner to regulate PMN transmigration. Mycoplasmal lipopeptide activated TLR2/6TLR2/6 potently inhibits PMN transmigration, highlighting a cross-talk mechanism by which CD47-mediated inhibition of PMN transmigration is dependent on TLR2 and MyD88, and that CD47 signaling enhances PMN sensitivity toward mycoplasmal lipopeptide recognized by TLR2 activation which, in turn, signals their arrival at a site of invasion and may facilitate antimicrobial function, acting as a fine-tuning microbial sensor by regulating TLR2 function and ultimately PMN transmigration and antimicrobial function. Further characterization of CD47-mediated PMN transmigration may prove to be useful in generating therapeutic strategies aimed at controlling acute inflammatory conditions as well as in other multiple cellular functions in which CD47 plays an important role, such as platelet activation (Chung et al. 1997; Dorahy et al. 1997), macrophage multinucleation (Han et al. 2000), immune cell apoptosis (Pettersen et al. 1999), DC maturation (Demeure et al. 2000), and as described a facilitator of microbial pathogen recognition (Lindberg et al. 1996; Liu et al. 2001; Parkos et al. 1996). In another study, LTA of *Streptococcus pneumoniae* and *S. aureus* activates immune cells via TLR2, LBP, and CD14, whereas TLR4 and MD2 are not involved (Schroder et al. 2003). However, to our knowledge, besides all present understanding of the TLR2/TLR6 heterodimer function and its pathway and coactivators, no PID has been described to be specifically related to this TLR heterodimer, CD47, and downstream signaling proteins.

TLR2, in Combination with TLR5, Recognizes Bacterial Flagellin

TLR5 is a receptor for flagellin, a protein that forms the PAMPs of the flagellum in flagellated bacteria (Hayashi et al. 2001). To date, no PID associated with this defect has been described in the literature. However, the presence of polymorphism on the gene encoding TLR5 and elevated risks of susceptibility to infections have become more frequent. The high and variable population frequencies of the polymorphism suggest that it may have a redundant role in host defense (Wlasiuk et al. 2009). It is frequently observed that mild phenotypes have been associated with the presence of polymorphisms on TLR5 and that, according to reports; it affects the control of only certain flagellated pathogens. Possible protective effects controlling the development of systemic lupus erythematosus and Crohn's disease have been reported in the presence TLR5 polymorphisms (Gewirtz et al. 2006; Hawn et al. 2005).

TLR4 Recognizes LPS of Gram-Negative Bacteria

Animal models with a defective TLR4 fail to produce TNF-α in response to the TLR4 ligand LPS and are more susceptible to infection with Gram-negative bacteria (Li and Cherayil 2004; Poltorak et al. 1998), and TLR4/TLR was associated with signaling and response to flagellin in syphilis (Mempel et al. 2007; Mizel et al. 2003). The importance of the NF-κB-dependent pathway of TLR signaling has been specifically demonstrated in mice lacking IL-1 receptor-associated kinase 4 (IRAK-4), which is required in TLRs signaling to activate IKK and induce NF-κB function. These animals demonstrate almost complete abrogation of TLR-induced functions (Suzuki et al. 2002). Similarly, mice deficient in NF-κB components also are unable to produce TNF-α in response to specific TLRs ligands (Weih et al. 1997); such deficiencies have allowed dissection of the pathway from TLRs to effector cytokine production. Human infectious susceptibility syndromes have also been directly correlated with inappropriate TLRs function. Most notably, a homozygous or compound heterozygous mutation in the gene encoding IRAK-4 results in recurrent pyogenic infection and completely abrogated TNF-α responses to TLRs ligands (Picard et al. 2003). Autosomal recessive IRAK-4 deficiency was first discovered in 2003 (Picard et al. 2003), and autosomal recessive MyD88 deficiency was first discovered in 2008 (von Bernuth et al. 2008). MyD88- and IRAK-4-deficient patients have homozygous or compound heterozygous mutations in *MYD88* or *IRAK4* gene, while heterozygous carriers are asymptomatic. All human TLRs other than TLR3 use both MyD88 and IRAK-4. Blood leukocytes from MyD88 and IRAK-4 deficient patients display a lack of IL-6 and CD62 ligand (CD62L) shedding on granulocytes following activation with TLRs-specific agonists. Both deficiencies confer a predisposition to severe pyogenic bacterial infection, presenting higher susceptibility to invasive infections (meningitis, sepsis, arthritis, osteomyelitis, and abscesses). The most common agents are *S. pneumoniae*, *S. aureus,* and *Pseudomonas aeruginosa*, mostly affecting the skin and upper respiratory tract, and necrozing infections are common (Picard et al. 2010, 2011). Since other measurable aspects of the innate and adaptive immunity are normal in this disorder, it presents a diagnostic challenge without the ability to evaluate TLRs function. Similarly, several other human genetic abnormalities associated with increased susceptibility to infection that also impair, or would be expected to impair, TLRs function have been described. These include mutations of the genes encoding TLR2, TLR4, and NEMO, and an NF-κB inhibitor, IκBα proteins (Orange et al. 2005). The latter two mutations can result in ectodermal dysplasia with immunodeficiency (EDA-ID), but in some cases the phenotype is limited to the

immunodeficiency of which TLRs functional impairment can be a feature (Orange et al. 2004). Furthermore, mutations of individual TLRs might be recognized only by testing the response to the specific cognate ligand, since they may not otherwise impact immune function. A key role for Brutons's tyrosine kinase (BTK) in NF-κB transactivation in response to LPS (Doyle et al. 2005) has been previously described, and also the ability of BTK to associate with the TIR of TLR4 has also been shown (Jefferies et al. 2003). X-linked recessive anhidrotic EDA-ID is caused by hypomorphic *IKBG/NEMO* mutation impairing NF-κB activation, where NEMO is a regulatory subunit of the IKK complex. An autosomal dominant (AD) form of EDA-ID was identified, caused by a hypermorphic heterozygous mutation of *NFKBIA/IKBA*, impairing the phosphorylation and degradation of IκBα and resulting in partial retention of NF-κB dimers in cytoplasm. Most NEMO-deficient patients lack IL-10 production in response to activation with TNF-α in whole-blood assay, impaired antibody response, in particular to glycans, hypogammaglobulinemia, and lower proportion of memory CD4 and CD8 T cells. Patients with IκBα and NEMO mutations present higher susceptibility to infections by presenting invasive pyogenic bacteria (*S. pneumoniae*, *Haemophilus influenzae*, *S. aureus*, *P. aeruginosa Klebsiella pneumoniae*, *Serratia marcescens*) causing meningitis, sepsis, arthritis, osteomyelitis and abscesses; atypical mycobacteria (*Mycobacterium avium*, *M. kansasii*); and in less proportion with parasites, viruses (HSV-encephalitis, adenoviral gastroenteritis, cytomegalovirus infection), and fungi (*Candida albicans*, *Pneumocystis jirovecii*) (Jouault et al. 2003; Netea et al. 2006; Orange et al. 2004, 2005; Picard et al. 2003; Tada et al. 2002).

Defects on Antiviral TLRs

There are two classes of intracellular pattern-recognition receptors that recognize virus: the RIG-I helicase receptors and TLRs. To our knowledge, no defects in the RIG-I helicase-receptor family are known. The TLRs that recognize viral PAMPs are TLR3 (double-stranded RNA), TLR7/8 (single-stranded RNA), and TLR9 (DNA) (Akira and Hemmi 2003; Akira et al. 2006; Kawai and Akira 2007; O'Neill 2008).

TLR3 Recognizes Double-Stranded RNA

TLR3 deficiency caused by mutations in TLR3 as well as mutation in UNC93B1, a protein in the TLR3 pathway (Casrouge et al. 2006), seems to be associated only with encephalitis caused by herpesvirus due to the fact that children with the deficiency have normal resistance to other pathogens such as bacteria and fungi (Zhang et al. 2007). The disease occurs mainly in early childhood (from 3 months to 6 years of age) during an initial infection with HSV-1 (Bousfiha et al. 2010; De Tiege et al. 2008). Recurrence of infections has been reported and suggests a role of TLR3 in affecting herpesvirus latency (Bousfiha et al. 2010). Inadequate levels of type I interferons may cause susceptibility to herpesvirus encephalitis, due to defects on signaling molecules in the type I IFN pathway (Chapgier et al. 2009; Dupuis et al. 2003). These clinical studies could possibly be explained by the fact that TLR3 can also be activated by synthetic double-stranded RNA (dsRNA), poly-riboinosinicribocytidylic acid (PIC) or polyinosinic-polycytidylic acid (poly(I:C)).

TNF receptor-associated factor 3 (TRAF3) has a function downstream of multiple TNF receptors in addition to induction of IFN-α, IFN-β, and IFN-λ production. This includes TLR3, which is deficient in some patients with HSV-encephalitis (HSE). Mice lacking TRAF3 die in the neonatal period, impossibiliting direct investigation of the role of TRAF3 in immune responses and host in vivo defenses. Autosomal dominant, human TRAF3 deficiency in a young adult with a history of HSE in childhood has been reported with loss of expression and loss of function of TRAF3 due to a dominant-negative mutant allele associated with impaired, but not abolished TRAF3-dependent responses upon stimulation of both TNF receptors and receptors that induce IFN production (Perez de Diego et al. 2010). An autosomal recessive form of complete TLR3 deficiency in a young man who developed HSE in childhood, but remained normally resistant to other infections has also been reported (Guo et al. 2011). This patient is compound heterozygous for two loss-of-function TLR3 alleles, resulting in an absence of response to TLR3 activation by poly(I:C) and related agonists in his fibroblasts. Moreover, upon infection of the patient's fibroblasts with HSV-1, the impairment of IFN-β and IFN-λ production resulted in high levels of viral replication and cell death. In contrast, the patient's PBMCs responded normally to poly(I:C) and to all viruses tested, including HSV-1. Consistently, various TLR3-deficient leukocytes from the patient, including $CD14^+$ and/or $CD16^+$ monocytes, plasmacytoid DCs, and in vitro derived monocyte-derived macrophages, responded normally to both poly(I:C) and HSV-1, with the induction of antiviral IFN production (Guo et al. 2011). Autosomal recessive (AR) UNC-93B and TLR3 deficiencies and AD TLR3 and TRAF3 deficiencies underlie HSE in some children. The literature also reports unrelated HSE children with AR or AD TRIF deficiency (Sancho-Shimizu et al. 2011). The AR form of the disease was found to be due to a homozygous nonsense mutation that resulted in a complete absence of the TRIF

protein. Both the TLR3- and the TRIF-dependent TLR4 signaling pathways were abolished. The AD form of disease was found to be due to a heterozygous missense mutation, resulting in a dysfunctional protein. In this form of the disease, the TLR3 signaling pathway was impaired, whereas the TRIF-dependent TLR4 pathway was unaffected. Both patients, however, showed reduced capacity to respond to stimulation of the DExD/H-box helicases pathway. To date, the TRIF-deficient patients with HSE described herein have suffered from no other infections. Moreover, as observed in patients with other genetic etiologies of HSE, clinical penetrance was found to be incomplete, as some HSV-1-infected TRIF-deficient relatives did not develop HSE (Sancho-Shimizu et al. 2011). These findings taken together suggest that TRAF3 deficiency is associated with a clinical phenotype limited to HSE, resulting from the impairment of TLR3-dependent induction of IFN. Based on the identification of genetic etiology for childhood HSE, indicating that TLR3-mediated immunity is essential for protective immunity to HSV-1 in the central nervous system (CNS) during primary infection in childhood, in at least some patients it is possible to suggest that human TLR3 is largely redundant for responses to double-stranded RNA and HSV-1 in various leukocytes, probably accounting for the redundancy of TLR3 for host defense against viruses, including HSV-1, outside the CNS. However, based on the description that human TRIF deficiency is also a genetic etiology associated with HSE, it is possible also to suggest that TRIF-dependent TLR4 and DExD/H-box helicase pathways are also largely redundant in host defense, demonstrating the importance of TRIF for the TLR3-dependent production of antiviral IFNs in the CNS during primary infection with HSV-1 in childhood.

The induction of IFN-β and/or IFN-λ1 in response to stimulation by the dsRNA analog polyinosinic:polycytidylic acid (poly(I:C)) was reported to be dependent on TLR3 and UNC-93B in all cells tested by Lafaille and collegues (Lafaille et al. 2012). However, induction of IFN-β and IFN-λ1 in response to HSV-1 infection was seen impaired selectively in UNC-93B-deficient neurons and oligodendrocytes. These cells were also much more susceptible to HSV-1 infection than control cells, whereas UNC-93B-deficient neural stem cells and astrocytes were not. TLR3-deficient neurons were also found to be susceptible to HSV-1 infection. The rescue of UNC-93B- and TLR3-deficient cells with the corresponding wild-type allele showed that the genetic defect was the cause of the poly(I:C) and HSV-1 phenotypes. The viral infection phenotype was rescued further by treatment with exogenous IFN-α or IFN-β (IFN-α/β), but not IFN-λ1. Thus, impaired TLR3- and UNC-93B-dependent IFN-α/β intrinsic immunity to HSV-1 in the CNS, in neurons and oligodendrocytes in particular, may underlie the pathogenesis of HSE in children with TLR3-pathway deficiencies (Lafaille et al. 2012). The description of two unrelated children with HSE carrying different heterozygous mutations (D50A and G159A) in TBK1, the gene encoding TANK-binding kinase 1, a kinase at the crossroads of multiple IFN-inducing signaling pathways, identified AD partial TBK1 deficiency as a new genetic etiology of childhood HSE, indicating that TBK1 plays an essential role in the TLR3- and IFN-dependent control of HSV-1 in the CNS (Herman et al. 2012). Both mutant TBK1 alleles are loss of function but through different mechanisms: protein instability (D50A) or a loss of kinase activity (G159A). Both are also associated with an AD trait, but by different mechanisms: haplotype insufficiency (D50A) or negative dominance (G159A). A defective polyinosinic-polycytidylic acid-induced TLR3 response was possible to be detected in fibroblasts heterozygous for G159A, but not for D50A TBK1. Nevertheless, viral replication and cell death rates caused by two TLR3-dependent viruses (HSV-1 and vesicular stomatitis virus) were high in fibroblasts from both patients, and particularly so in G159A TBK1 fibroblasts. These phenotypes were rescued equally well by IFN-α2b. Moreover, the IFN responses to the TLR3-independent agonists and viruses tested were maintained in both patients' PBMCs and fibroblasts. The narrow, partial cellular phenotype thus accounts for the clinical phenotype of these patients being limited to HSE (Herman et al. 2012).

Other studies highlight the role of TLR3 in vaccinia infection (Harte et al. 2003; Lai and Gallo 2008). In addition to innate immunity, dsRNA is also capable of boosting adaptive immunity against viral infection, for example, by cross-priming viral-specific $CD8^+$ T cells (Salio and Cerundolo 2005; Seya et al. 2003, 2006). Indeed, TLRs ligands are used as adjuvants for the formation of an effective adaptive immune response. Among the CNS cells, both microglia and astrocytes of rat, mouse, and human origin have been extensively studied for TLR-mediated activation (Bsibsi et al. 2002; Carpentier et al. 2008; McKimmie and Fazakerley 2005) and it has been shown that TLR3 is the predominant TLRs on astrocytes that mediates activation of antiviral, as well as inflammatory genes (Bsibsi et al. 2006; Carpentier et al. 2005; Farina et al. 2005; Rivieccio et al. 2005). In addition, treatment of astrocytes with poly(I:C) elicits a strong antiviral activity (Rivieccio et al. 2006; Suh et al. 2007) and also it has been shown that microglia express all known TLRs and are the only cell type that can effectively respond to LPS in the CNS (Laflamme and Rivest 2001) and robustly to both PIC (TLR3 ligand) and LPS (TLR4 ligand) (Carpentier et al. 2008; Jack et al. 2005; Town et al. 2006). Activation of TLR3 or TLR4 recruits the TIR domain-containing adapter

TRIF which phosphorylates IRF3 through the activation of recently identified IRF3 kinases, TBK1, and IKKε (also called IKKi) (Fitzgerald et al. 2003; Sharma et al. 2003). The recruitment of TRIF to TLR3 or TLR4 also activates the canonical IKKs (α, β, and γ), which then activate NF-κB and MAP kinases. NF-κB, MAP kinases, and IRF3 are all required to activate human *IFN-β* gene (Enoch et al. 1986; Lenardo et al. 1989). Secreted IFN-β then activates the Jak/Stat pathway in an autocrine manner resulting in the transcriptional induction of IFN-stimulated genes. These properties of microglia along with the presence of other cell surface receptors render them highly reactive to a variety of innate and adaptive immunological stimuli (Kreutzberg 1996; Perry and Gordon 1988; Streit et al. 2005). Activated microglia induce proinflammatory cytokines and reactive oxygen radicals that have been shown to cause death of neurons and oligodendrocytes in a number of experimental systems (Cai et al. 2003; Chao et al. 1992; Lehnardt et al. 2002). However, microglial activation does not always lead to neurodegeneration, as microglia can also generate growth factors (including brain-derived neurotrophic factors and insulin-like growth factors) and anti-inflammatory cytokines that can contribute to neuroprotection (Gordon 2003; Hanisch and Kettenmann 2007; Schwartz et al. 2006). In addition, there is evidence that proinflammatory cytokines and LPS can elicit an antiviral program in microglia (Lokensgard et al. 1997; Si et al. 2004).

TLR7, in Combination with TLR8, Recognizes ssRNA

TLR7 and TLR8 sense ssRNA from viruses or host ribonucleoproteins and synthetic imidazoquinolines such as R848, whereas TLR9 senses unmethylated CpG motifs in viral and bacterial DNA and in host DNA. An endogenous interaction between BTK and human TLR8 and TLR9 in the monocytic cell line THP1 has been reported (Doyle et al. 2007) revealing that R848, ssRNA, and CpGB-DNA activate BTK in THP1 cells as shown by phosphorylation of the tyrosine 223 residue of BTK and also by increased autokinase activity, demonstrating a requirement of BTK for NF-κB activation, and participating in the pathway by increasing phosphorylation of p65 on serine 536 activated by TLR8 and TLR9 (Doyle et al. 2007). BTK, a cytoplasmic non-receptor tyrosine kinase, is a member of the Tec family of protein kinases. It was first identified in the male immune disorder X-linked agammaglobulinemia (XLA). The XLA syndrome is manifested by severe defects in early B cell development, resulting in a nearly complete absence of peripheral B cells and immunoglobulins of all classes (Bradley et al. 1994). Various hemizygous mutations in the human *BTK* gene were identified as being responsible for the XLA phenotype (Hashimoto et al. 1996). These include mutations in both the kinase domain and the pleckstrin homology domain, which affect kinase activity and translocation to the membrane, respectively. The pleckstrin homology domain is important in the process of BTK activation since it localizes BTK to the membrane through its interaction with phosphatidylinositol 3,4,5-triphosphate. In mice, a naturally occurring mutation in the pleckstrin homology domain has been mapped to arginine 28 (R28C), which causes X-linked immune deficiency (Xid) in these mice, not as severe as in humans, because B cells of mice use other kinase like BTK, called Tec. Mutated BTK in Xid mice is prevented from associating with the membrane, thus inactivating it (Park et al. 1996).

TLR9 Recognizes DNA

The demonstration that PBMCs from patients with XLA that have dysfunctional BTK are impaired in the induction of interleukin-6 by CpG-DNA establishes BTK as a key signaling molecule that interacts with and acts downstream of TLR8 and TLR9 (Doyle et al. 2007) by interacting with TIR domains. BTK-deficient mice have exhibited high susceptibility to acute mouse adenovirus type-1, and XLA patients are highly susceptible to enteroviral infections (Moore et al. 2004; Winkelstein et al. 2006). Both TLR8 and TLR9 have been shown to be expressed in B cells, and their activation has been shown to induce antibody production and proliferation (Chuang and Ulevitch 2000; Dasari et al. 2005; Schlaepfer et al. 2006); however, the lack of functioning BTK in XLA patients downstream of TLR8 and TLR9 might explain the susceptibility of XLA patients to viral infections (Doyle et al. 2007). Heme oxygenase (HO)-1 has also been shown to exert immunomodulatory functions via cell type-specific anti-inflammatory effects in myeloid/macrophage cells. It has been demonstrated that BTK is involved in the upregulation of *HO-1* gene expression via TLR signaling in macrophages. The specific BTK inhibitor LFM-A13 blocked HO-1 induction by the classical TLR4 ligand LPS in cell cultures of RAW264.7 monocytic cells and primary mouse alveolar macrophages. Moreover, upregulation of *HO-1* gene expression was abrogated in LPS-stimulated alveolar macrophages from BTK2/2 mice. This induction was mediated by the transcription factor Nrf2, which is a master regulator of the antioxidant cellular defense. Accordingly, nuclear translocation of Nrf2 in LPS-treated macrophages was reduced by BTK inhibition. BTK-dependent induction of HO-1 gene expression was also observed upon macrophage stimulation with ligands of TLR2, TLR6, TLR7, and TLR9, suggesting that BTK is

required for *HO-1* gene activation by major TLRs pathways (Vijayan et al. 2011). Another study regarding another PID demonstrated that B cells from common variable immunodeficiency (CVID) patients show a defective response to direct TLR9 specific stimuli and also to stimulation with *S. pneumoniae* and *H. influenza* extracts, suggesting that this poor response could also contribute to the hypogammaglobulinemia and impaired vaccine response present in CVID patients (Escobar et al. 2010). Although acting through different pathways, neither CpG-DNA nor anti-CD40 and IL-21 stimulation are able to induce B cells from CVID patients to produce normal levels of immunoglobulins, and this defect is not corrected by the addition of anti-IgM. This may imply a signaling defect in both pathways or alternatively indicate that B cells from CVID patients die in culture after stimulation. This would suggest that B cells from CVID patients have a generalized response defect to different stimuli, which may underlie the hypogammaglobulinemia and the poor vaccine responses characteristic of these patients (Clemente et al. 2011). B cell and plasmacytoid DC activation was induced via TLR9, on which CpG-DNA did not up-regulate the expression of CD86 on CVID B cells even when costimulated by the B cell receptor, or induce production of IL-6 or IL-10 as it does for normal B cells. TLR9, found intracytoplasmically and on the surface of oligodeoxynucleotide-activated normal B cells, was deficient in CVID B cells. TLR9 B cell defects were not related to the proportions of $CD27^{+}$ memory B cells. CpG-activated CVID plasmacytoid DCs (PDCs) did not produce IFN-γ in normal amounts, even though these cells contained abundant intracytoplasmic TLR9 and no mutations of TLR9 were found. These demonstrate that there are broad TLR9 activation defects in CVID which would prevent CpG-DNA-initiated innate immune responses; these defects may lead to impaired responses of plasmacytoid DCs and loss of B cell function (Cunningham-Rundles et al. 2006). Although the result of TLR9 dysfunction on PDC-driven immune responses is less clear, PDC contributes most of the type I IFN produced in viral infections (Barchet et al. 2005). As a result, one would hypothesize that TLR9 defects might lead to viral infections; however, these are not especially characteristic of CVID (Cunningham-Rundles and Bodian 1999; Hammarstrom et al. 2000; Hermaszewski and Webster 1993). Recent studies show that mice lacking the TLRs adapter My88 have only a partial reduction in IFN responses and normal viral resistance, indicating that there are TLR-independent pathways to IFN production which may compensate for TLRs defects and permit effective viral immunity (Barchet et al. 2005; Hochrein et al. 2004). MyD88 is an adapter molecule that transduces signals from TLRs receptors (with the exception of TLR3) and from the receptors for IL-1 and IL-18 (Adachi et al. 1998; Takeuchi et al. 2000). The signaling involves a cascade of protein kinases, including the serine–threonine kinase IRAK-4 (Akira and Takeda 2004). Studies in the past decade have identified germline mutations in IRAK-4 (Cardenes et al. 2006; Chapel et al. 2005; Comeau et al. 2008; Davidson et al. 2006; Ku et al. 2007; Medvedev et al. 2003; Picard et al. 2003; Szabo et al. 2007) and MyD88 (von Bernuth et al. 2008) in patients with increased susceptibility to pyogenic bacteria and *Pseudomonas* species. These defects in the TLR-IL-1 receptor pathway were found in young patients with recurrent, severe pneumococcal infections. The lymphocytes of one of the first reported patients had a poor response, in vitro, to stimulation with endotoxin and IL-1 (Kuhns et al. 1997). The invasive infections associated with these mutations are usually meningitis and septicemia caused by *S. pneumoniae* and *S. aureus* and less frequently by *P. aeruginosa* and *Salmonella* species. *S. aureus* is the main cause of localized infection, but *P. aeruginosa* and *S. pneumoniae* can also cause this complication (Bousfiha et al. 2010). Invasive infections begin to appear in infancy, with a cumulative mortality of 30–40 % (Ku et al. 2007). Children with an MyD88 or IRAK-4 deficiency, by contrast, do not have major infections when they reach adulthood (Bousfiha et al. 2010). This difference suggests that the development of protective T cell- and B cell-mediated immunity after infancy compensates for the defective TLR-IL-1 receptor pathway (Ku et al. 2007). Other studies, however, show the recognition of herpes simplex viruses by TLR2 and TLR9 in DCs (Sato et al. 2006) and TLR7 and TLR9 in *verruca* and *molluscus contagiosum* (Ku et al. 2008).

TLR10, the Recent Member of the TLR Family

TLR10 is the most recently identified human homolog of the Drosophila Toll protein, with the gene localized to chromosome 4p14, and the specific ligands and functions of TLR10 are currently under investigation. Sequence analysis predicted that the 811-amino acid protein, with 50 % of similarity to TLR1 and TLR6, contains a signal peptide, multiple leucine-rich repeats, a cysteine-rich domain, a transmembrane domain, and a cytoplasmic TIR domain. Uzma Hasan and co-workers in 2005 determined that the human and rat TLR10 genes both contain a single exon, and by genomic sequence analysis, they determined that the TLR10 gene was clustered with TLR1 and TLR6, two receptors known to function as coreceptors for TLR2, on chromosome 4p14 (Hasan et al. 2005). Signaling studies showed that TLR10 required MYD88, but not TIRAP, TRAM, or TRIF, to activate immune system promoters. TLR10 deletion may suppress the formation of secondary

structures specifically formed by TLR10, which can be potentially important for signaling, and suggests that unknown cytosolic factors and/or transcription factors may depend on the presence of nonconserved TLR10 sequences (Hasan et al. 2005). Multiple alternatively spliced transcript variants which encode different protein isoforms have also been found for the *TLR10* gene. This TLR is predominantly expressed in immune cell-rich tissues, such as spleen, lymph node, and lung (Chuang and Ulevitch 2001). Its exact role and PID associated with its defect have not been described in the literature until present moment, although there are reports of its association with other nosological disturbances like prostate cancer risk (Stevens et al. 2008), childhood IgA nephropathy (Park et al. 2011), Crohn's disease (Abad et al. 2011; Morgan et al. 2012), sarcoidosis (Veltkamp et al. 2012), urothelial bladder cancer (Guirado et al. 2012), susceptibility to graft-versus-host disease (Sivula et al. 2012), trophoblast apoptosis (Mulla et al. 2013), and Meniere's disease (Requena et al. 2013).

Screening of Patients with TLR Dysfunction and Immunodeficiency

The clinical features of defects in pattern-recognition receptors are usually due to disturbed cytokine homeostasis, either decreased cytokine production and increased susceptibility to infections (in the case of TLR3 or MyD88 deficiency) or overwhelming release of cytokines (in the case of NLRP3 defects). The clinical presentation ranges from severe (in cases of MyD88 and IRAK-4 deficiencies) to mild (in cases of MBL and TLR5 deficiencies). The clinical severity is greatest in infancy but decreases thereafter, perhaps because adaptive immune responses that develop after infancy compensate for the ineffective innate immunity. By contrast, the level of severity of classic PID usually persists throughout life (WHO 1997). In 2006, Deering and Jordan developed a reproducible assay to evaluate TLRs function for the absence of a single or multiple TLRs responses by measuring TNF-α production in PBMC supernatants in an enzyme-linked immunosorbent assay. The approach uses ligands for TLRs 1/2, 2/6, 3, 4, 5, 6, 7, and 9 stimulation and is dependent on gene transcription and NF-κB activation. Since then, it has been applied to clinical use for screening patients with PID affecting TLRs function, with a history of recurrent or severe infection, directing the additional genetic-molecular diagnosis. All human TLRs other than TLR3 use both MyD88 and IRAK-4; blood leukocytes derived from MyD88- and IRAK-4-deficiency patients display a lack of IL-6 production by whole blood or a lack of CD62L shedding from granulocytes following activation with most of the TLRs and IL-1R agonists tested, with the exception of agonists of TLR3, which uses an MyD88- and IRAK-4-deficiency pathway (Deering and Orange 2006; von Bernuth et al. 2006). This by itself could also be likely to reveal previously unappreciated genetic defects that result in infectious susceptibility and is particularly useful because many of these patients do not present otherwise measurable impairments of immune function. This assay was validated on three patients with a mutation in the *IKBKG* gene, encoding NEMO protein, and values found were almost uniformly below the standard deviation of healthy donors for all ligands tested. NEMO-deficient patients generally display a lack of IL-10 production in response to activation with TNF-α in whole-blood assays. Other parameters included an impaired antibody response, in particular that to glycans, including pneumococcal capsules and severe impairment of T cell proliferation in response to anti-CD3 (Deering and Orange 2006). The clinical and immunological TLRs dysfunction phenotypes can also be evaluated based on the TLR responses in individual leukocyte subsets, TLR responses for multiple cytokines and phosphorylated MAP kinases p38, c-JUN N-terminal kinase and extracellular signal-regulated kinase are a possibility of TLR responsiveness evaluation. Laboratory assays for TLR activity evaluation may also include PBMC cytokine (IFN-α, IFN-β, IFN-ω, and TNF-α) production after in vitro stimulation with specific agonist on culture overflow (Guo et al. 2011; Xu et al. 2012), NF-κB cytosol to nucleus translocation (Picard et al. 2011; Yang et al. 2012; Zheng et al. 2012), flow cytometry for CD62L shedding, and p65 NF-κB subunit cytosol to nucleus translocation after IKKγ dissociation (Maguire et al. 2011; Wang et al. 2010). NF-κB p65 subunit translocation studies can also be made using immunofluorescence microscopy. Gene reporter assays are frequently used as means to evaluate NF-κB function in consensus DNA-binding sequences.

Conclusions and Future Perspectives

TLRs are a family of transmembrane receptors that have been preserved throughout evolution and which selectively recognize a broad spectrum of microbial components and endogenous molecules released by injured tissue. Each TLR recognizes specific PAMPs, and the identification of these ligands by TLRs triggers signaling pathways which lead to the expression of numerous genes involved in a defensive response. In mammals, the products of these genes initiate inflammation, coordinate the effector functions of innate immunity, instruct and modulate adaptive immunity, and initiate tissue repair and regeneration. Genetic variations greatly influence immune responses

toward pathogenic challenges and disease outcome. Different mutations and experimental models which alter TLR function have revealed the significance of these receptors in susceptibility to infection and their involvement in the pathogenesis of a large number of non-infective inflammatory disorders. TLRs are present in the plasmatic membrane (TLR1, TLR2, TLR4, TLR5, TLR6) and endosome (TLR3, TLR7, TLR8, TLR9) of leukocytes, and to initiate the innate immune response, TLRs associate with cytoplasmic adapter proteins through TIR domain interactions. The four principal signaling adapter proteins include MyD88, MAL, TRIF, and TRAM, and the fifth protein SARM, involved in negative regulation of TLR pathways, is usually considered a part of the TIR domain-containing adapter protein group. TLR pathways and the adapter proteins are associated with a number of diseases, including infection, sepsis, inflammatory, allergic and autoimmune diseases, and cancer. The classical proinflammatory TLR signaling pathway leads to the synthesis of inflammatory cytokines and chemokines, such as IL-1β, IL-6, IL-8, IL-12, and TNF-α. In humans an impaired signaling of the TLRs pathway has been established, with dominant infectious phenotype of patients in the occurrence of pyogenic bacterial infections, virus and fungi. The clinical and biological phenotypes of human TLRs deficiencies define a novel group of PID characterized by a selective and profound defect of TLRs signaling pathway, with weak development of inflammatory signs leading to severe and higly susceptible to invasive bacterial infections, in major part located in the skin and upper respiratory tract. The same occur with MyD88 and IRAK-4 deficiencies, defined as a novel group of PID characterized by a selective and profound defect of the TIR canonical signaling pathway. In contrast with these immunodeficiencies, hypomorphic NEMO deficiency is associated with susceptibility to various bacteria, mycobacteria, fungi and viruses, and delay in the development of inflammatory signs is common. However, we must have in mind that TLRs are not the only players in inflammation, and several important questions remain unanswered such as the mechanism behind the cooperation of different TLRs and their interaction with the different PRRs, accessory cells, and immunological mediators to trigger an optimal immune response. Currently, TLRs are viewed as important targets for the development of new vaccines and innovative therapies to prevent and treat human diseases. However, the therapeutic modulation of TLR function could cause as consequence unexpected harmful responses related to non-TLR inflammatory signals that are not considered. This raises the need to be more precise in understanding the role of each TLR in the pathophysiology of the different diseases. Consequently, the success of potent biological TLR-based therapies prescribed under an individual basis will require new techniques to reach diagnosis and efforts of multidisciplinary teams including immunologists with detailed knowledge of potential side effects.

Conflict of interest All authors declare no conflict of interest.

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