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REVIEW

Toll-Like Receptors and Their Adapter Molecules

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Abstract—Toll-like receptors (TLR) are among key receptors of the innate mammalian immune system. Receptors of this family are able to recognize specific highly conserved molecular regions (patterns) in pathogen structures, thus initiating reactions of both innate and acquired immune response finally resulting in the elimination of the pathogen. In this case every individual TLR type is able to bind a broad spectrum of molecules of microbial origin characterized by different chemical properties and structures. Recent data demonstrate the existence of a multistep mechanism of the TLR recognition of the pathogen in which, in addition to receptors proper, the involvement of different adapter molecules is necessary. However, functions of separate adapter molecules as well as the principles of formation of a multicomponent system of ligand-specific recognition are still not quite understandable. We describe all identified as well as possible (candidate) adapter TLR molecules by giving their brief characteristics, and we also propose generalized possible variants of the TLR ligand-specific recognition with involvement of adapter molecules.

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Toll-like receptors (TLR) are able to recognize certain highly conserved molecular patterns (pathogen-associated molecular patterns (PAMP)) in microbial structure thus leading to development of innate and acquired immune response [1]. Molecules of different chemical nature (proteins, sugars, lipopolysaccharides (LPS), lipopeptides, glycopeptides, etc.) play the role of PAMP [2]. In this case the same type PAMP have similar structure among the wide spectrum microorganisms, which allows the latter to use a limited set of pattern-recognizing TLR for recognition of total variety of pathogens (bacteria, viruses, fungi, etc.).

Recently, an increasing amount of data is appearing that prove the ability of TLR to bind both exogenous PAMP and structurally different molecules of alarmins of endogenous origin [3]. Alarmins include heat shock proteins, HMGB1, HDGF, S100 proteins, urea, etc. During different processes associated with cell damage (inflammation, tumor progression) the concentrations of alarmins sharply increase in intercellular space, thus signaling the development of pathology. Due to the association of alarmins with microbial tissue damage, the above-described group of molecules is also called the family of damage-associated molecular patterns (DAMP).

The recent discovery of numerous new molecules recognized by TLR has resulted in the fact that at present the existence of over 50 endogenous and exogenous TLR ligands of different chemical nature and origin have been proved (Table 1). So an increasing amount of data

Abbreviations: AP-1, activator protein 1; CCR6, chemokine receptor 6; DAMPs, damage-associated molecular patterns; dsRNA, double-stranded RNA; HDGF, hepatoma-derived growth factor; HMGB1, high mobility group protein 1; IFNα, interferon α; IRF, interferon regulatory transcription factor 1; LBP, LPS-binding protein; LRR, leucine rich repeats; LTA, lipoteichoic acid; MBP, mannose-binding protein; MyD88, myeloid differentiation primary response gene (88); NALP (NACHT-, LRR-, and PYD-containing proteins); NF-κB, nuclear factor kappa-light-chain-enhancer of activated B cells; NK cells, natural killer cells; PAMPs, pathogen-associated molecular patterns; PGRP, peptidoglycan-recognizing protein; PRRs, pattern-recognizing receptors; RAGE, renal cell carcinoma antigen; RP105, radioprotective protein with molecular mass 105 kDa; SP-A, surfactant-associated protein A; SP-D, surfactant-associated protein D; TIR, Toll/interleukin-1 receptor homology domain; TLRs, Toll-like receptors; TNFα, tumor necrosis factor α; TNP, Tamm–Horsfall glycoprotein; TRAM, TRIF-related adaptor molecule; TRIF, TIR-domain-containing adapter-inducing interferon-β.

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Table 1. Ligands recognized by TLR

Structural component	Organism	Source
1	2	3
	TLR1/2/6	
Glycolipids	*Treponema maltophilum*	[4]
Glycosylphosphatidylinositol (GPI-anchor)	*Toxoplasma gondii, Trypanosoma cruzi*	[5, 6]
Lysophosphatidylserine	*Schistosoma mansoni*	[7]
Lipoteichoic acids	*Staphylococcus aureus, Streptococcus pneumoniae*	[8]
Lipoarabidomannan	*Mycobacterium avium, M. tuberculosis, M. chelonae, M. kansasii, M. bovis*	[9-11]
Lipophosphoglycans	*Leishmania major*	[12]
Proteins		
Tc52	*T. cruzi*	[13]
porin	*Neisseria meningitidis, Haemophilus influenzae*	[14, 15]
modulin	*Staphylococcus epidermidis*	[16]
V-antigen	*Yersinia pestis, Y. pseudotuberculosis, Y. enterocolitica*	[17]
A-protein of outer membrane	*Klebsiella pneumoniae*	[18]
seeligeriolysin	*Listeria seeligeri*	[19]
Lipopolysaccharides	*Leptospira interrogans, Porphyromonas gingivalis, Legionella pneumophila, Flavobacterium meningosepticum, Prevotella intermedia, Escherichia coli, Shigella flexneri*	[20-24]
Glycoproteins		
glycoprotein PGP	*P. intermedia*,	[25]
hemagglutinin	measles virus	[26]
Peptidoglycans	*S. aureus*	[27]
Lipopeptides/lipoproteins	*Borrelia burgdorferi, Treponema pallidum, Mycoplasma fermentans, Mycoplasma pneumoniae, Mycoplasma arthritidis, S. aureus*	[28-33]
Glycolipoprotein 19 kDa	*M. tuberculosis*	[34]
Lipid A	*F. meningosepticum*	[35]
Soluble factor	*N. meningitidis*	[36]
Proteins of virus envelope	herpes simplex virus, chicken-pox virus, human cytomegalovirus, measles virus	[37-39]
Zymosan	*Saccharomyces cerevisiae*	[40]
Hyaluronan	*Mus musculus*	[41]

Table 1. (Contd.)

1	2	3
HMGB1	*Mus musculus*	[42]
β-Defensin-3	*Homo sapiens*	[43]
Heat-shock proteins		
HSP70	*M. musculus*	[44, 45]
GP96	–"–	[46]
Surfactant protein SP-D	–"–	[47]
	TLR3	
dsRNA	RNA-containing viruses	[48]
	TLR4	
Mannan	*S. cerevisiae*	[49]
Glucuronoxylomannan	*Cryptococcus neoformans*	[50]
Glycosylphosphatidylinositol (GPI-anchor)	*T. cruzi*	[51]
Glycolipids	*Treponema brennaborense*	[4]
F-protein	respiratory-syncytial virus	[52]
Envelope proteins	murine mammary gland tumor virus	[53]
Lipopolysaccharide	Gram-negative bacteria	[54]
Surfactant proteins		
SP-A	*M. musculus*	[55]
SP-D	–"–	[48]
β-Defensin-2	*Gallus gallus, H. sapiens*	[56, 57]
Taxol	*Taxus brevifolia*	[58]
Heat-shock proteins		
HSP60	*H. sapiens*	[59]
HSPB8	–"–	[60]
Hyaluronan	*G. gallus*	[61]
Fibronectin	*H. sapiens*	[62]
Fibrinogen	–"–	[63]
HMGB1	*Sus* ssp.	[42]
Saturated fatty acids	animal and vegetable fats	[64]

Table 1. (Contd.)

1	2	3
Low density lipoproteins	*H. sapiens*	[65]
Seeligeriolysin	*L. seeligeri*	[19]
Flavolipin	*F. meningosepticum*	[66]
ER-112022, E5564, E5531	synthetic components	[67]
Heparin	*M. musculus*	[68]
	TLR5	
Flagellin	bacterial flagellates	[69]
	TLR7	
ssRNA	*M. musculus*	[70, 71]
Bropyrimin	synthetic components	[72]
Guanosine analogs	–"–	[73]
	TLR8	
ssRNA	RNA-containing viruses	[71]
R-848	synthetic component	[74]
	TLR9	
CpG DNA	any bacteria	[75]
Chromatin–IgG complexes	*H. sapiens*	[76]
	TLR11	
Unknown component	uropathogenic bacteria	[77]
Profilin-like protein	*T. gondii*	[78]

are appearing that show that recognition by a cell of various molecular patterns in most cases is a complex multistage process in which TLR are not the only participants.

In fact, at present many molecules have been identified that are involved in TLR ligand recognition. For some of these molecules the mechanism of their involvement in the recognition process itself has also been determined [79]. Most likely, just formation of a multicomponent mechanism of molecular pattern recognition with involvement of different adapter molecules provides for high sensitivity of the innate immune system as well as for multispecificity of TLR themselves towards the recognized ligands. However, presently available information on functioning of some adapter TLR molecules is highly divergent. Besides, there is no generalized idea concerning the ways of formation of the TLR recognition specificity system for the whole variety of identified ligands.

The aim of this review is to generalize the available information concerning functions of different adapter molecules in recognition of different molecular patterns as well as to create a combined scheme of interactions between TLR, adapter molecules, and ligands with regard to the role of each class of molecules in PAMP and DAMP recognition.

TLR, TLR-RECOGNIZED LIGANDS, AND LOCALIZATION

The discovery in mammals at the end of 1990s of the *Drosophila melanogaster* Toll-receptor homolog (further called TLR4) involved in development of endotoxin (LPS-induced) shock in animals and defining the sensitivity of animals to Gram-negative bacteria led to the first step in determination of the molecular mechanisms of pathogen recognition and activation of the innate immune system [80, 81].

Later several different families of innate immune system receptors were identified that are able to recognize the presence of a pathogen by binding certain highly conserved molecular patterns (PAMP) within structural components of microorganisms. According to their functions, the described receptors were called pattern-recognizing receptors (PRR). Using a limited set of PRR, immunocompetent cells are able to detect the presence of different pathogenic organisms and their structural components as well as (after PAMP binding to PRR) to launch protective mechanisms of both innate and acquired immunity aimed at the elimination of the pathogen. The TLR is among the best-studied PRR. Receptors of this family have the most extended list of ligands including practically any biological molecules of different chemical nature and structure such as fatty acids, lipids, proteins and peptides, lipopeptides, sugars, liposaccharides, glycopeptides, nucleic acids, and many others.

Now 11 types of human TLR have been identified, each of which is able to recognize its own, differing from others, ligand spectrum, and some types of receptors (TLR2, TLR4) bind numerous exogenous and endogenous molecules of different chemical properties and structure (Table 1).

TLR are expressed on cytoplasmic membranes and in subcellular compartments (endosomes) by many cell types including those of non-hematopoietic and mesenchymal origin. Each above-mentioned cell type expresses its own particular set of TLR. Cells of hematopoietic origin such as macrophages, neutrophils, and dendritic cells (DC) express a broader TLR spectrum, also with some distinctions between different cell types. For example, there are distinctions in the expressed TLR repertoire even among subtypes of dendritic cells (plasmacytoid and conventional) [82]. Most likely, such selectivity in TLR expression by different cell types is defined by cell localization within an organism and thus by probability and frequency of encountering a particular pathogen.

STRUCTURE–FUNCTION CHARACTERISTICS OF TLR

Structurally TLR are single-stranded transmembrane glycoproteins consisting of N-terminal extracellular domain (contains leucine-rich repeats (LRR) responsible for ligand binding), transmembrane part containing one α-helix, and C-terminal intracellular TIR domain (Toll/IL-1-homologous domain that was so-called due to the structural similarity with intracellular domain of IL-1 receptor).

The TIR domain is present in the structure of many animal and plant transmembrane and cytoplasmic proteins [83, 84], most of which are directly involved in functioning in the immune protection of the organism. The main function of the TIR domain is establishment of noncovalent protein–protein bonds for activation of signal transduction into the cell.

After ligand molecule binding to extracellular TLR domains, adjacent receptors dimerize, which results in change in conformation of the intracellular TIR domains, which, in turn, results in emergence of binding sites for TIR-containing adapter molecules (TRIF, TRAM, MyD88) whose recruiting and activation launch the cascade of reactions that finally results in activation of a number of transcription factors (AP-1, NF-κB, IRF) (Scheme 1) [85].

The above-mentioned transcription factors alter expression of many genes whose products are involved in cell reactions to the presence of a microorganism or its structural molecules. Among molecules whose expression depends on TLR activation there are anti-inflammatory cytokines and chemokines; molecules remodeling cytoskeleton; molecules involved in development of apoptosis of eukaryotic cells; various antimicrobial peptides, and soluble PRR. Besides, direct involvement of TLR in activation and differentiation of dendritic cells as well as of B and T lymphocytes has been shown [86].

Now numerous data have accumulated that prove the key role of TLR in development of reactions of both innate and acquired immune system against microbes.

ANALYSIS OF INTERACTION OF LIGANDS WITH LRR OF EXTRACELLULAR TLR DOMAIN

LRR are known to be present in structures of ~6000 proteins including ~300 human proteins [87]. The presence of LRR, providing for ligand–receptor interaction, is necessary for many receptors of the immune system (NOD receptors, NALP receptors, TLR, etc.). The LRR domain consists of 19-25 LRR of conserved motifs, each of which consists of 20-30 amino acids arranged in the sequence L(X2)LXL(X2)NXL(X2)L(X7)L(X2), where X is any amino acid. The tertiary structure of each LRR contains one α helix and one β sheet joined by a loop. The LRR is separated from the transmembrane part by the so-called LRR-carboxy-terminal domain, which is also characterized by its specific sequence

CXC(X23)C(X17)C. Combined LRR of the extracellular domain form a horseshoe-shaped structure and the ability of TLR to interact with different ligands is associated just with this structure.

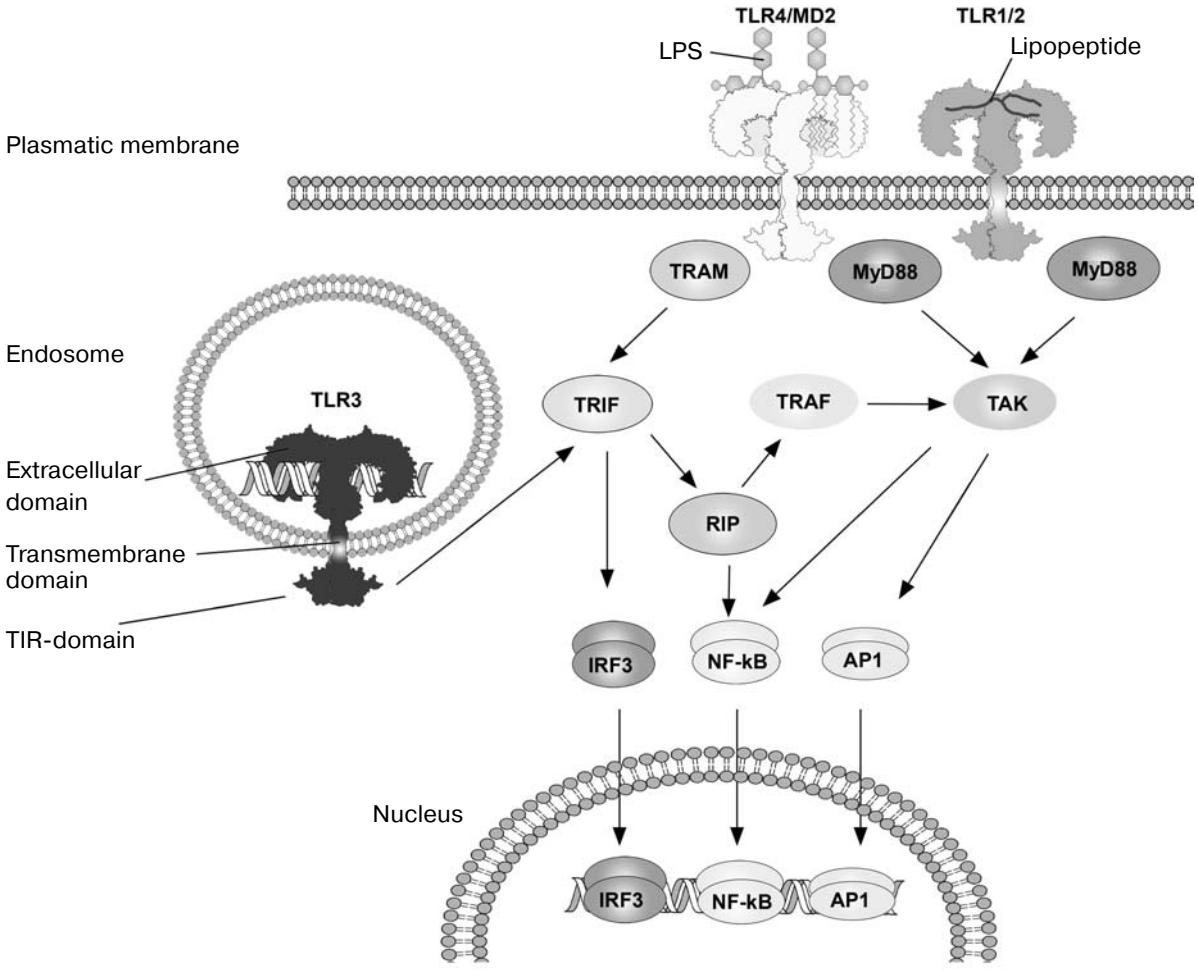
To date the structures of four TLR complexes with their own ligands have been obtained by X-ray analysis: TLR1–TLR2–Pam3CSK4 (triacylated bacterial lipopeptide) [88], TLR3–viral double-stranded RNA (dsRNA) [89], TLR4–MD2–erythoran (synthetic analog of LPS lipid A) [90], as well as TLR4–MD2–LPS [91].

This information is necessary for investigation of more precise mechanism of the TLR ligand recognition, which will allow the next step in understanding the functioning of the innate immune system.

Analysis of data on TLR functioning makes it possible to divide all TLR into two main groups. Receptors of the first group (TLR3, 5, 7, 8, 9) are able, using LRR of the extracellular domain, to interact independently with recognizable molecules, thus forming functionally competent ligand–receptor complexes (Scheme 2a). In this case ligands of the first TLR group are biological molecules of some particular class (TLR3, 7, 8, 9 ligands are nucleic acids, TLR5 ligands are proteins).

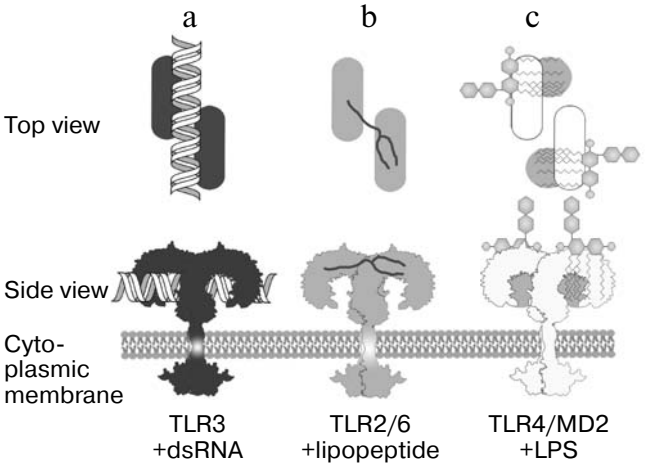
The other molecules, including compounds with complex chemical nature (lipopeptides, LPS, etc.), form the ligands of the second TLR group (TLR1, 2, 4, 6). The above-mentioned receptors are characterized by involvement of additional coreceptor molecules in the process of ligand recognition. Different types of TLR (like formation of heterodimers TLR1/2, TLR2/6) (Scheme 2b) and various adapter molecules (like formation of TLR4–MD2–LPS complex) (Scheme 2c) can act as additional molecules.

Attraction of different TLR types into the recognition process makes it possible to introduce changes in total ligand-recognizing surface of receptor complex that,



TLR-initiated signal cascade. TLR1/2 heterodimer activation is able to activate only the MyD88-dependent signal pathway, whereas activation by TLR4 initiates both MyD88- and TRIF-dependent signal pathways. The MyD88-dependent signal pathway results in activation of NF-κB and AP1 transcription factors, whereas the TRIF-dependent pathway leads to IRF3 activation. Recognition of TLR3 within cellular endosomes results in TRIF-dependent activation of IRF3

Scheme 1



Variants of TLR ligand recognition. a) Ligand (dsRNA) recognition is provided by the TLR3 homodimer forming the ligand-recognizing surface common for two receptor molecules. b) Ligand (lipopeptide) is recognized by heterodimer of TLR2 and TLR6 forming a common ligand-recognizing surface. c) Two molecules, TLR4 and adapter molecule MD2, holding an LPS molecule near the receptor, are involved in ligand (lipopolysaccharide) recognition

Scheme 2

in turn, influences specificity of recognition of molecules of a particular chemical nature or structure. In this case adapter molecules are able to present the caught molecule "correctly" to the receptor complex. In the case of recognition of biological molecules with ambiguous chemical nature, an adapter molecule interacts with the ligand part not recognized by the given TLR type, simultaneously presenting the corresponding highly conserved ligand site necessary for recognition to the receptor surface.

Thus, when the active complex TLR4–MD2–LPS forms, five of six lipid chains of LPS are hidden in the hydrophobic pocket of the MD2 molecule, while the other lipid chain and regions of the hydrophilic part interact with TLR4. Note that molecules with a smaller oligosaccharide part (lipid IV_A and lipid A synthetic analog eritoran, which are not able to establish bonds with TLR4) act as antagonists of this receptor. Thus, interaction with just a small region of LPS is necessary for activation of TLR4. In this case the described interaction cannot proceed without correct ligand presentation in the absence of adapter molecule MD2.

In addition to direct proof of the participation of the MD2 molecule in ligand–receptor complex formation together with TLR4, there are now data that indicating the existence of coreceptor functions in other adapter molecules as well (like CD14, CD36), the presence of which within the same lipid raft together with TLR on the surface of plasma membrane was shown.

All the above-mentioned examples show that TLR are not the only molecules involved in the process of PAMP and DAMP recognition.

ADAPTER MOLECULES AND THEIR ROLE IN LIGAND RECOGNITION TOGETHER WITH TLR

In addition to MD2, there are now many identified molecules functioning as intermediaries between TLR ligands and receptors proper (Table 2). It was also shown that TLR able to recognize independently their own ligands (TLR3, 5, 7, 8, 9) also have their own adapter molecules. In this case the latter fulfill a number of important functions in the innate immune system without involvement in the actual process of ligand recognition.

All presently known adapter molecules can be divided into three groups according to their functions. Molecules of the first group are components of the plasma membrane in the immediate vicinity of TLR and are involved in formation of functionally active ligand–receptor complex (such as the MD2 molecule).

The main task of the second group molecules, also anchored at the plasma membrane, is concentration of ligand near the receptor complex and ligand presentation to the first group adapter molecules or directly to TLR. Most likely molecules of the second group include CD14, CD36, and RAGE, for which association with the TLR-containing receptor complex is shown along with the ability to enhance activation signal and increase receptor complex sensitivity.

The third group is formed by soluble adapter molecules present in the extracellular space (including those in blood and in lung surfactant). Soluble adapter molecules recognize PAMP and DAMP and deliver bound compounds to cell surfaces, which makes possible, under conditions of the macroorganism's large dimensions compared to those of microorganisms, to increase the macroorganism's immune system sensitivity to the developing pathogen invasion. The system of DAMP- and PAMP-binding carriers also allows more rapid launching of the immune response system reactions upon the particular pathogen's entry into the body. The LPS-binding protein (LBP), mannose-binding protein (MBP), peptidoglycan-recognizing proteins (PGRP), etc. can be considered as soluble adapter molecules.

Thus, adapter molecules can fulfill different functions during recognition of TLR ligands. In this case the appropriate particular set of adapter molecules is used for each individual TLR combination with the recognized ligand.

Now, after generalization of available information, we shall describe the role of each separate adapter molecule in the system of TLR ligand recognition. For convenience we divide by convention all presently known adapter molecules according to their arrangement relative to the eukaryotic cell into two groups, soluble and membrane-bound, although some adapter molecules are able to exist within the organism simultaneously in the two forms.

Table 2. TLR adapter molecules involved in DAMP recognition

DAMP-recognizing adapter molecules	Recognized DAMP name	Tissue specificity/ cell types expressing adapter molecule	Interaction with TLR or other adapter molecules	References
Collectins				
SP-A	mannose, L-fucose, galactose, glucose, rough type LPS	lungs/Clara cells, type II alveolar cells	sCD14, TLR2, TLR4, and MD2	[92-94]
SP-D	maltose, L-fucose, mannose, glucosamine, rough type LPS	–"–	TLR2, TLR4, and sCD14	[47, 93]
MBP	sugars with 3- and 4-hydroxyls in pyranose ring	blood	sCD14	[95]
Antimicrobial peptides				
β-Defensin-2	negatively charged molecules	skin, respiratory tract	TLR4	[56, 96]
LL37	DNA	bone marrow, testes/ keratinocytes, neutrophils	TLR9	[97]
Alarmins				
HMGB-1	–"–	all cells	RAGE	[98, 99]
RAGE	–"–	–"–	TLR9	[98, 99]
Membrane-bound molecules				
CD36	diacylglycerides, LTA	thrombocytes, erythrocytes, monocytes, epithelial cells of mammary glands and skin	TLR2/6	[100]
CD14	smooth LPS	macrophages, dendritic cells (DC), neutrophils	TLR4	[101]
	dsRNA		TLR3	[102]
RP105	lipoproteins	B cells, macrophages, DC	TLR2	[103]
Various				
LBP	LPS	blood	mCD14	[104]
B/L cathepsins	DNA	all cells	TLR9	[105]
MD2	only lipid A	immunocompetent cells	TLR4	[106]
PGRP-SA*	lysine type peptidoglycans	hemolymph	Toll	[107]
PGRP-SD*	–"–	–"–	–"–	[108]
GNBP3*	β-1,3-glycans	absent	–"–	[109]
GNBP1*	lysine type peptidoglycans	–"–	–"–	[109]

* Shown for *Drosophila melanogaster*.

SOLUBLE ADAPTER MOLECULES

Soluble pattern-recognizing molecules. *Collectins SP-A, SP-D, and MBP.* Surfactant-associated proteins A and D (SP-A, SP-D) are present in lung surfactant and are an important component of the innate immune system. Surfactant-associated proteins belong to the collectin class of the C-type lectin superfamily and contain in their structure a carbohydrate-binding domain that provides for collectin binding to sugars, both isolated and pathogen surface-associated [110].

It was also shown that both proteins are able to bind specifically LPS of the rough type bacteria [111, 112]. For SP-A its interaction was shown separately with the main membrane protein of type A *Haemophilus influenzae* outer membrane [113], capsular polysaccharide of *Klebsiella* [114], 120-kDa surface glycoprotein of *Pneumocystis jirovecii* [115], while for SP-D the possibility of interaction with lipids of *M. pneumoniae* and lipoarabidomannan of *M. tuberculosis* and *M. avium* was shown [116-118]. Lung collectins also interact with many viruses via recognition by its carbohydrate-binding domain of mannosylated N-terminal carbohydrates of type A influenza virus hemagglutinin and neuraminidase, of the respiratory syncytial virus G protein, etc. [119, 120].

In addition to direct PAMP recognition, carbohydrate-binding domains of surfactant-associated proteins are involved in interaction with TLR2,4 and adapter molecule CD14 [47, 92-94], while SP-A protein is also involved in association with the MD2 molecule.

It should be determined which functions are fulfilled by lung collectins during TLR ligand recognition. However, now there are data concerning ambiguous effects of lung collectins on TLR activation. It was shown that SP-A is able to inhibit the secretion of the anti-inflammatory cytokine TNFα by alveolar macrophages in response to addition of peptidoglycan and zymosan [92], whereas addition of the rough type LPS in the presence of SP-A enhances secretion of anti-inflammatory cytokines [94]. Another representative of collectins, mannose-binding protein (MBP) that recognizes peptidoglycan [121], LPS of *Salmonella enterica* [122], and lipoarabidomannan of *M. avium* [123], is able to interact directly with the peptide part of CD14 coreceptor [95] without using the lectin-binding domain. Besides, different researchers have also shown that MBP is able to form complexes with TLR, thus enhancing the activation signal cascade launched upon lipoteichoic acid recognition by TLR2/6 heterodimer.

Peptidoglycan-recognizing proteins. PGRP have been identified in most kinds of animals including insects, mollusks, and vertebrates [124]. PGRP comprise a group of highly conserved molecules that recognize peptidoglycan molecules of Gram-positive and Gram-negative bacteria. These proteins are present both in soluble (all mammalian PGRP) and transmembrane (some insect PGRP) forms. Extracellular secreted PGRP of drosophila (PGRP-SA and PGRP-SD) activate Toll-receptor after binding to peptidoglycan [107, 108]. In this case the presence of PGRP-SA is necessary for Toll-receptor activation and signal cascade launching, whereas PGRP-SD just enhances the activation signal. There are still no data concerning the involvement of mammalian PGRP in TLR activation, but PGRP functions as PAMP-binding molecules and scavenger-receptors are considered as proved.

Antimicrobial peptides. *Cathelicidin LL37.* It has recently been shown that antimicrobial protein LL37 of the cathelicidin class is able in the norm to bind its own endogenous DNA by forming a complex leading to TLR9 activation and following IFNα secretion by dendritic cells [97]. This property of LL37 is apparently necessary for additional stimulation of innate immunity in response to development of damage processes in the cell. According to present-day concepts, the ability to form immunogenic complexes with endogenous DNA defines the key role of antimicrobial protein LL37 in development of autoimmune diseases, including psoriasis.

These data allow may answer the question whether TLR9 is able to recognize isolated dsDNA, or whether its activation requires formation of a protein–nucleic acid complex.

β-Defensin. Defensins are evolutionarily conservative positively charged proteins forming a large group of antimicrobial peptides [125]. Direct involvement of these proteins in functioning of the innate immune system of a macroorganism explains prevalent expression of these proteins by epithelial and immunocompetent cells (neutrophils, monocytes, macrophages). Antimicrobial effect of defensins stems from their ability to form pores in membranes of microorganisms, which results in their immediate death. These proteins enhance chemotaxis of immature dendritic cells and memory T cells via interaction with chemokine receptor CCR6 [126]. Also, it was shown that mouse β-defensin activates TLR4, thus leading to maturation of immature dendritic cells [56, 96], while human β-defensin-3 activates mononuclear cells of peripheral blood due to binding to TLR1 and TLR2 [127].

The data proving the interaction of defensin with different molecules (adhesins of *Porphyromonas gingivalis* [128], influenza A virus with surfactant-associated protein SP-D [129], HIV envelope glycoprotein gp120, coreceptor molecule CD4 [130]) as well as the absence of resolved structure of defensin complex with TLR leave unanswered the question whether defensins play the role of direct TLR ligands or they are adapter molecules helping to form receptor complexes for binding different molecules.

Tamm–Horsfall glycoprotein. Polymeric Tamm–Horsfall protein (THP) is expressed in the large ascending canaliculus of Henle's loop of kidney (30-50 mg/day)

and is one the major urinary proteins [131]. Mechanisms of involvement of Tamm–Horsfall protein in functioning of the mammalian innate immunity system are still not fully understood, but Pak et al. [132] noted the ability of this protein to bind uropathogenic *E. coli* with subsequent inhibition of the ability of the bacteria to adhere to epithelial cells. The inhibitory activity of THP against different bacteria (*K. pneumoniae*, *S. saprophyticus*) was also shown. Thus, inflammatory processes caused by these bacteria in THP-knockout mice was more pronounced and accompanied by significant leukocyte infiltration and development of fibrosis [131].

THP also exhibits some immunomodulating properties: it induces TNF secretion by human monocytes, enhances neutrophile chemotaxis and phagocytosis [133], and activates dendritic cells by increasing expression of MHC molecules. Several receptors have been identified for Tamm–Horsfall glycoprotein including TLR4 and scavenger receptors SR-AI, Cla-1, and SREC-I [134, 135]. It was shown that initiation by THP of dendritic cell maturation takes place just via activation of the TLR4-dependent signal cascade.

Due to interaction with TLR4 and simultaneous ability to bind bacterial components, THP acts as a candidate adapter TLR molecule. In this case a hypothesis is suggested concerning scavenger receptor involvement as additional adapter coreceptor molecules.

Alarmin HMGB1. Nucleosomal nuclear protein (high-mobility group box 1 protein (HMGB1)) exists in practically all cell types [136], and its functions in the norm are coupled with stabilization of the nucleosomal structure of DNA and with regulation of expression of some genes [137]. However, it has been recently shown that HMGB1 also acts as a classical alarmin [3].

During the development of inflammation, the appearance of HMGB1 in the intercellular space can be due to two processes: passive release of cells, dying in a non-apoptotic way, and to directed secretion in response to anti-inflammatory stimulation by different types of immunocompetent cells (activated macrophages, natural killer cells (NK cells)).

After release into the intercellular space, HMGB1 carries out some immunomodulating functions; it enhances chemotaxis of monocytes, macrophages, dendritic cells [138, 139], endothelial, and smooth muscle cells [140, 141], induces maturation of dendritic cells (myeloid and plasmacytoid) [142, 143], and recruits stem cells and stimulates their proliferation [144].

There are data showing that extracellular HMGB1 is capable of direct interaction with at least three receptors (TLR2, TLR4, RAGE), thus activating transcription factor NF-κB in neutrophils and macrophages [43]. Due to DNA binding ability (including that for alien DNA), HMGB1 forms complexes with DNA, which also interacts with TLR9, a different TLR type. Receptor RAGE, binding HMGB1–DNA complex on the cell surface, is also involved in recognition. It was shown that involvement of HMGB1 protein and RAGE receptor in DNA TLR9 recognition not only enhances but simultaneously accelerates cellular cytokine response [99]. In this case just combined addition of HMGB1 and DNA induced IFNα and TNFα production in dendritic cells [98].

LPS-binding protein (LBP). LBP is the acute form protein carrying out two functions upon binding to bacterial LPS. At low LPS concentrations LBP defines high sensitivity and enhances the microorganism's reaction to endotoxin. Thus, mice with LBP gene knockout exhibited lower resistance to peritoneal infection by *S. typhimurium* [145], *E. coli* [146], and to lung infection by *K. pneumoniae* [147]. The described function is carried out due to the LBP ability to bind and extract LPS monomers from bacterial membranes [148, 149] with subsequent highly processive LPS transport (up to 150 molecules per minute) to coreceptor LPS-recognizing molecule CD14 molecule [150].

At high LPS concentrations LBP prevents development of septic reactions via inhibition of developing immune response by LPS transport to the high-density serum lipoproteins [151, 152] or by direct involvement in formation of LBP–LPS aggregates for following excretion of excess LPS from organism [153].

MEMBRANE-ASSOCIATED ADAPTER MOLECULES

Coreceptor molecule CD14. CD14 is the pattern-recognizing glycoprotein molecule consisting, in particular, of 11 LRR whose functions are binding bacterial LPS in complex with LBP [154] and subsequent presentation of a bound molecule to signal TLR4–MD2 complex. CD14 can be present both in membrane-bound (mCD14) and soluble (sCD14) forms. The CD14 soluble form is necessary for monomeric LPS transfer to mCD14 [155] or directly toTLR4/MD2 receptor complex if cells do not express mCD14 [154, 156].

It was shown that CD14 significantly enhances formation of TLR4–MD2–LPS complex on the cell surface [157, 158] and enhances the following TLR4-mediated cell response to the presence of LPS [159]. Mice with *cd14* gene knockout were less sensitive to LPS and more resistant to endotoxic shock [160]. Besides, the CD14 molecule is able to enhance cell response by binding molecules whose chemical nature differs from that of LPS, such as lipopeptides, lipoteichoic acid, lipoarabidomannan, peptidoglycan, as well as dsRNA [102], and by presenting these molecules to TLR2, 1, 6, 3. In this case different sites on CD14 are necessary for stimulation of TLR2- and TLR4-mediated signaling [161].

There are also several identified sites in CD14 structure whose alterations do not influence the LPS-binding ability of CD14, but they cancel the subsequent transduc-

tion of activation signal into the cell [162, 163]. The above-described regions might be necessary for attraction of additional molecules involved in the transduction of the activation signal.

MD2 molecule. MD2 is the soluble secretory protein that binds the LPS presented by CD14 [164], and due to interaction with extracellular TLR4 domain it forms the activating complex TLR4–MD2–LPS. Recently the quaternary structure of TLR4–MD2 complex with ligands eritoran and lipid IV_A has been determined, which confirmed direct involvement of the MD2 molecule in formation of the ligand–receptor complex [90, 165].

The necessity of the participation of MD2 in both LPS recognition and subsequent TLR4-mediated cell response was also proved in experiments *in vivo*. MD2-knockout mice do not response to LPS stimulation and are able to survive after endotoxic shock, but they exhibit higher sensitivity to infection by *S. typhimurium* [166].

A minimal sequence of 15 amino acids was identified in the primary structure of MD2 protein that is responsible for binding of both LPS and lipoteichoic acid [167]. Also, it was shown that antitumor preparations taxol and lipid IV_A (lipid A precursor) are able to activate mouse, but not human, TLR4–MD2 complex [168, 169]. Akashi et al. [169] showed that replacement of mouse MD2 by human MD2 in complex with mouse TLR4 completely abrogates the activating effect of lipid IV_A. The data show that MD2 is able to define specificity in molecular recognition by TLR4–MD2 complex. However, there are data proving that TLR4 itself is also able to form specificity of ligand recognition [170, 171].

There are some works that prove the role of MD2 as a chaperon for TLR4 that provides for necessary glycosylation and intracellular transport [172, 173].

Receptor complex RP105–MD1. The high structural similarity between RP105 and TLR made it reasonable to combine the above-described molecules in one subfamily [174, 175]. RP105, like TLR, is a transmembrane protein whose extracellular domain contains leucine-rich repeats (LRR). However, its intracellular part consists of just 6-11 amino acids retaining the ability of RP105 to interact with intracellular signal molecules [176]. Now the expression of RP105 by myeloid cells including monocytes, macrophages, and dendritic cells as well as by B lymphocytes has been shown [174, 177]. In this case the surface expression of a given molecule depends on association with soluble MD2 homolog MD1.

The direct interaction of RP105 with any endogenous or exogenous ligand is still unproved. However, there are data suggesting the involvement of RP105–MD1 in TLR-mediated cell response. Thus, it was shown that proliferative and humoral immune response to the addition of LPS was decreased in RP105- and MD1-deficient mouse B lymphocytes compared to control wild-type cells [178, 179]. RP105-expressing macrophages and dendritic cells after LPS induction, on the other hand, reacted to a lower level of cytokine expression compared to RP105-deficient cells [174]. Finally, the interaction of RP105–MD1 complex with TLR4–MD2 as well as inhibition of LPS binding to TLR4–MD2 without immediate RP105–MD1 recognition by LPS was proved experimentally using 293 transfected cells [174, 180].

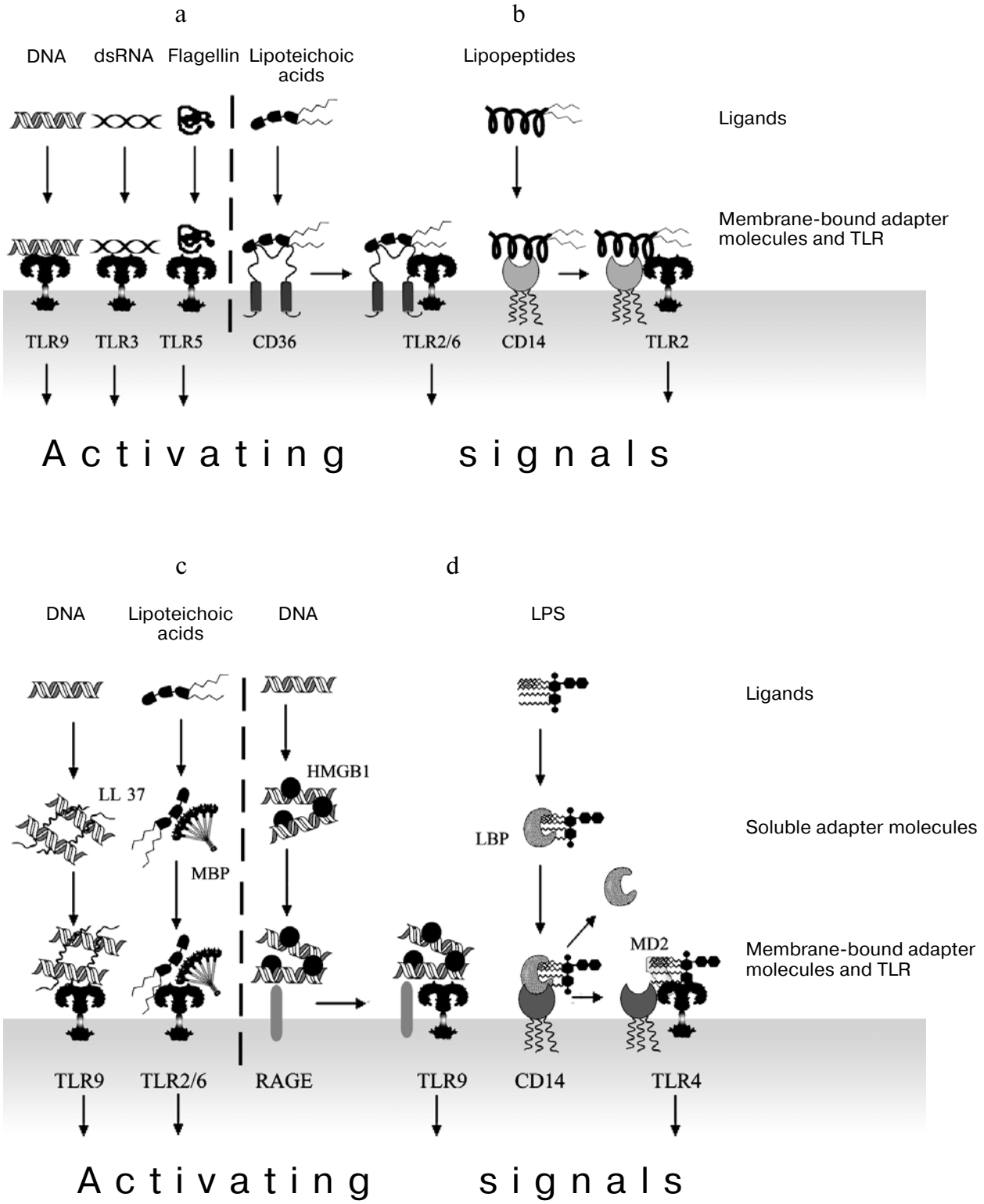
Besides, RP105–MD1 is also an adapter molecule of TLR2. RP105 interacts with TLR2 and thus enhances TLR2-mediated activation of macrophages of mice infected by *M. tuberculosis* [103].

Scavenger receptor CD36. This is a surface transmembrane protein twice penetrating the plasma membrane of eukaryotic cells. CD36 is expressed by many cell types including macrophages, thrombocytes, adipocytes, endothelial, and epithelial cells [181].

CD36, class B scavenger receptor, recognizes several pathogen-associated molecular structures such as lipoteichoic acid (LTA), diacyl lipopeptides (MALP2) [100], and fungal β-glucan [182]. It was shown that the TLR2/6 activation induced by LTA and diacyl lipopeptides is much dependent on the presence of the CD36 molecule. In addition, data of Hoebe et al. [100] are indicative of the ability of CD36 to serve as TLR2 coreceptor. Further studies have shown that after addition of diacylglycerides, CD36, being within lipid rafts on the surface of plasma membrane, is able to establish contact with TLR2/6 heterodimer [183]. Perhaps such association plays an important role in formation of intracellular activation signal in response to the presence of the above-described bacterial molecules in the intercellular space. It was also shown that CD36-deficient mice were more sensitive to *S. aureus* infection compared to the wild-type mice [184]. These investigations support the necessity of involvement of CD36 in formation of full-value innate immunity.

There are now numerous molecules that are identified as being involved in recognition of TLR of the whole variety of ligands. Acting as intermediates (adapters) between recognized ligands and TLR, they fulfill an important function in correct functioning of the innate immune system.

All presently known TLR adapter molecules can be divided to two large groups by their localization in the eukaryotic cell. The first group consists of pattern-recognizing molecules secreted by the macroorganism's cells into border extracellular spaces like blood plasma and lung surfactant (MBP, SP-A, SP-D, LBP, PGRP, etc.). The main function of such molecules is recognition of molecular patterns and delivery of bound compounds to TLR-expressing cells. Soluble pattern-recognizing molecules are the first to detect the presence of molecular components of the pathogen or endogenous signal molecules (alarmins) by launching innate immunity reactions and simultaneously providing for the macroorganism's high sensitivity to infectious invasion. Taking into account the huge dimensions of macroorganisms compared to the size of pathogens, functioning of such mole-



Possible mechanisms of DAMP recognition and TLR activation. a) DAMP is directly recognized by TLR thus leading to activation of the latter. b) Initially DAMP is recognized by an adapter molecule localized on the eukaryotic cell membrane, which then presents bound ligand to TLR, thus activating the latter. c) Primary DAMP recognition is provided by soluble adapter molecules in the extracellular space after which the soluble adapter molecule presents the bound ligand to TLR, activating the latter. d) Primary DAMP recognition is provided by soluble adapter molecules in the extracellular space after which the soluble adapter molecule presents the bound ligand to the membrane-bound adapter molecule localized on the eukaryotic cell membrane, which in turn presents DAMP to TLR, thus activating the latter

Scheme 3

cules becomes especially important for initiation of the reactions of the innate immunity system. Besides, it was shown [97, 98] that complexes of soluble adapter molecules LL37 and HMGB1 with endogenous DNA acquire the ability to cause significantly stronger TLR9 activation. This phenomenon can be indicative of involvement of soluble adapter molecules in regulation of functioning of the macroorganism by intensifying the immune system reaction to combinations of potentially dangerous stimuli such as damages of its own cells (emergence of endogenous DNA) and detection of pathogens within the macroorganism (secretion of microbial proteins, HMGB1).

The molecules of the second group are present on the surface of the eukaryotic cell plasma membrane (CD36, mCD14) or exist in soluble form in the immediate vicinity of its surface (sCD14, MD2). The list of functions of such molecules includes recognition of a ligand bound by molecules of the first group, concentration of the ligand next to the receptor complex (this property together with the ability for soluble pattern-recognizing molecules to deliver bound ligand to receptor complex makes it possible to create near the receptor complex local high concentration of the ligand, which might be important in receptor signal enhancement), ligand presentation to receptor complex, and TLR activation after interaction of the adapter molecule with the ligand.

The above-mentioned functions necessary for launching the full-value TLR-mediated immune response are distributed between several adapter molecules involved in the recognition process. Thus, in some cases ligand can make successive contacts with a set of adapter molecules before its recognition by the TLR-containing complex.

Analysis of available data allowed us to try to systematize possible variants of ligand recognition by different pattern-recognizing molecules including ligand interaction with TLR proper. As a result, the overall scheme of TLR activation can be represented in the form of four recognition variants, four of which suggest involvement of different adapter molecules (Scheme 3).

TLR are able to recognize independently some ligands by launching an intracellular signal cascade after interaction. An example of such recognition scheme can be the proved direct interaction of TLR3 with dsRNA [48]. Most likely, the same principle exists in the interaction of TLR5 with the bacterial protein flagellin.

The second type of recognition requires participation of membrane-bound adapter molecules that interact on the cell surface with soluble ligand with subsequent presentation of the latter to TLR. There are some data suggesting that bacterial lipopeptides and lipoteichoic acid first bind coreceptor molecules CD36 and CD14, and then they are presented to the TLR2/6 complex [100, 185].

Realization of the third mechanism of TLR ligand recognition requires participation of soluble adapter molecules. In this case expression of appropriate adapter molecules enhances TLR-dependent cell response upon addition of TLR ligands. Examples of such activation pathway are formation of DNA complex with antimicrobial protein LL37 or with HMGB and subsequent interaction with TLR9 [97, 98].

The last variant of TLR activation by ligand requires the participation of both soluble and membrane-bound adapter molecules. In this case the ligand is first recognized by a soluble adapter molecule (like LBP, sCD14 for LPS, HMGB1 for DNA), after which the ligand is transferred onto coreceptor present on the cell membrane (mCD14, MD2, and RAGE, respectively) [98, 186]. In the last step membrane-bound adapter molecule presents bound ligand to TLR (TLR4 or TLR9).

Evidently, just the use of adapter molecules allowed TLR (especially TLR2 and TLR4), having a highly conserved LRR ectodomain homologous among different types, to recognize the declared ligand variability.

The proposed schemes of TLR ligand recognition, using a large set of adapter molecules each of which fulfills definite functions, allow the organism to recognize a broader spectrum of molecules of different chemical nature, to detect the presence of PAMP and DAMP molecules at lower concentrations, and to react more rapidly to emergence of PAMP and DAMP.

Of course, adapter molecules are also not highly specific, but it is possible that just the concrete combination of adapter molecules, recognizing any ligand type, defines specificity of the total recognition cascade.

Besides, it should be recognized that presently identified adapter molecules cannot completely explain the mechanism of recognition of the whole diversity of DAMP and PAMP TLR, which leaves open the question concerning still unidentified mechanisms of TLR-mediated recognition of ligands with supposed involvement of different coreceptor or adapter molecules.

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