RESEARCH STUDIES

Toll-like receptors

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Abstract

The study results of the last 10-12 years have established that the activity of the nonspecific immunological protection system is based on a limited set of specific membrane receptors, which have been called molecular 'pattern recognition receptors' (PRRs) that recognize pathogen associated molecular patterns (PAMPs). After the detection of PAMPs PRRs trigger an inflammatory response that leads to the destruction of pathogens. Toll-like receptors (TLRs) refer to PRRs, along with three other families: NOD-Like receptors, RIG-I-Like receptors and C-type Lectin receptors. In humans, TLRs are present on dendritic cells, macrophages, neutrophils, B-lymphocytes, mast cells, enterocytes, some cells of the central nervous system (astrocytes), hepatocytes, etc. The attempts have been made in order to obtain vaccines against microbes, as well as the vaccines designed to trigger or enhance an anti-tumor immune response by stimulating the TLRs and other similar receptors. The suppression or enhancement of TLRs activity is an important pathogenic mechanism, underlying many infectious or immunological diseases (including autoimmune). These mechanisms can be used for the further elaboration of new methods and algorithms for prevention, diagnosis and treatment of the mentioned diseases. Thus, not only strong fundamental data on the mechanisms of the innate immunity have been accumulated and the attempts to apply these results have been made, but also it has been managed to integrate the non-specific immunity mechanisms with the adaptive immunity and to found a new field in immunology – an integrated immunology, which creates the background for new therapeutic, diagnostic and preventive strategies in the future.

Key words: toll-like receptors, molecular pattern, pathogens.

Toll-подобные рецепторы

Nowadays, the "nonspecific resistance of the body" concept is fundamental when relating to the intimate molecular mechanisms of the development of the body's primary response to the harmful actions of various pathogens such as bacteria, viruses, fungi, etc. In mammals, the nonspecific resistance is directly involved in the activation and subsequent orientation of the adaptive immune response. Thus, a unique body protection system against various pathogens is generated.

The innate immunity mechanism has been preserved throughout the evolution, its function being quick and efficient fight against the pathogens penetrating the body. The nonspecific immune protection system primarily does not recognize the individual antigens specific to certain pathogens, but it recognizes a string of common structures characteristic of many representatives of a group of such organisms. This type of recognition is based on a limited set of specific membrane receptors, which have been called molecular 'pattern recognition receptors' (PRRs) that recognize pathogen associated molecular patterns (PAMPs). The discovery of the PRRs, involved in the production and development of the body response reaction to the pathogen invasion, is one of the latest, fundamental achievements [2]. The presence of PRRs is a proof of the structural and metabolic "economy" principle, which is characteristic of living organisms. Thus, the mammalian cells, possessing a limited number of receptors with a defined area of recognition, may specifically apprehend and bind a significantly higher number of pathogens due to the ability of recognizing joint molecular patterns of their antigens. As a result, the spectrum and the number of the pathogens, against which the cell and the whole body are protected, significantly increases, such a major response being achieved with the relatively modest resources.

Substances with different structures, properties and origin, such as lipopolysaccharides (LPS) of gram-negative bacteria, peptidoglycan (PGN) of gram-positive bacteria, double-stranded RNA (dsRNA) of viruses, β-glycans of fungi and others refer to typical PAMPs. These structures are found in germs (bacteria, viruses, fungi, parasites), but not in mammals. After their detection, PRRs trigger an inflammatory response leading to the destruction of the pathogens.

The Toll-like receptors (TLRs) belong to the PRRs category that initiates a pro-inflammatory signaling pathway together with the other three categories of PRRs – NOD-Like receptors, RIG-I-Like receptors and C-type Lectin receptors. The TLRs family comprises a significant number of phylogenetically related proteins present in both vertebrates and invertebrates. TLRs, specific to the organisms that are at various stages of evolutionary development, show both similarities and differences in the structure, conformation and properties that determine the peculiarities of their activation mechanisms and the accomplishment of the specific intracellular response [17].

The Toll-like receptors are named so because of the similarity to the protein encoded by the Toll gene present in *Drosophila*. Jules Hoffmann (Nobel Prize, 2011) has proved that the Toll gene does not only control the dorso-ventral pattern during embryogenic development of *Drosophila* but is also involved in the immune defense of the fly. The protein produced by the Toll gene is an important transmembrane

receptor for the antifungal immunity of the adult *Drosophila* without which it can not survive a fungal infection [13, 14, 19].

Currently about 15 different forms of TLRs are attested. In humans, this type of receptors is found on dendritic cells, macrophages, neutrophils, B-lymphocytes, mast cells, enterocytes, cells of the central nervous system (astrocytes), hepatocytes, etc. Some cells are characterized by an individual set of TLRs, which determines the specific response of the cell to the activation of this set of receptors. Thus, TLR1 is expressed in all types of leukocytes, including monocytes, polymorphonuclear leukocytes, T and B lymphocytes and NK cells; TLR2, TLR4 and TLR5 are found on cell membranes of the monocytic series; TLR3 is found in human dendritic cells, etc. [16]. The area of cellular distribution of TLRs allowed their division by the following categories:

1. Ubiquitous or pervasive in most of the cells (TLR1).
2. Restricted to a limited number of cells (TLR2, TLR4 and TLR5).
3. Specific for a particular type of cells (TLR3) [15] (fig. 1).

All the representatives of the TLRs family are transmembrane proteins of type 1 that possess two common structural domains for all individual TLRs crucial for the achievement of their functions: the extracellular domain (N-teminal) containing repetitive sequences rich in leucine (leucine-rich repetitive domain), a single transmembrane helix and cytoplasmic domain (C-terminal) – TIR (Toll/IL-1 receptor), similar to the cytoplasmic domain of the receptor family for IL-1.

The above mentioned structural elements are directly involved in the recognition and binding of specific PAMPs, and in the transfer of information within the cell with the induction of the immune and metabolic responses [8, 15].

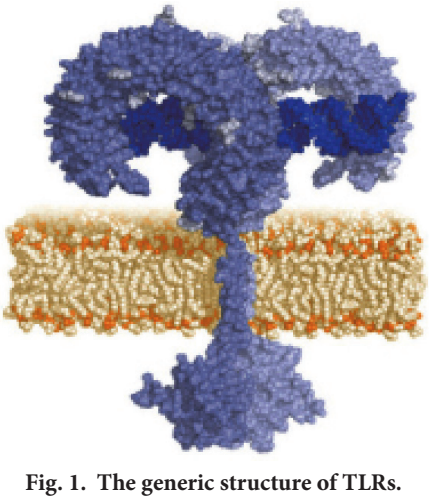

Fig. 1. The generic structure of TLRs. (http://www.invivogen.com/review-tlr).

The TLRs family members are able to recognize certain pathogens and to orchestrate a corresponding response of the nonspecific and adaptive immunity due to the capacity of identifying PAMPs common to various microorganisms that invade the human body. The lipoproteins with triacylated N-terminal cysteine are recognized by TLR1, which forms a heterodimer with TLR2, whereas diacylated lipoproteins are recognized by TLR6 in cooperation with TLR4; the double-stranded RNA specific to viruses is recognized by TLR3; TLR4 may specifically bind lipopolysaccharide; TLR5 recognizes flagellin; the members of TLR7-TLR9 subfamily recognize viral or bacterial single-stranded RNA, as well as compounds with cyclic elements in their structures, such as the heme or the nucleic acids (CpG sequences of the bacterial DNA) (tab. 1) [10, 17]. Despite the wide variety of ligands recognized by the TLRs, all receptors have leucine-rich repetitive regions and after the binding of the ligand, the extracellular domains form a dimmer in the form of „m".

The TLRs studies have established two features of the ligand recognition mechanism, which allow the identification of a significantly large number of ligands by a relatively limited number of Toll-like receptors.

Some TLRs possess the ability to recognize the same structural pattern, common to many pathogens, which the latter cannot modify easily and quickly.

The same TLRs can recognize several common structural patterns of the pathogen. For example, TLR4 recognizes not only lipopolysaccharides, but also a number of viral glycoproteins.

Recent studies have revealed the existence of the endogenous ligands of TLRs. The heat shock proteins (HSPs) physiologically involved in the folding, assembly and the transport of proteins are produced in increased amounts in stressful situations. Some specific forms of HSPs - HSP60 and HSP70, eliminated by the necrotized cells, activate the inflammatory process with the help of TLRs. The TLR4 induces tissue damage, the inflammatory process being activated by HMBG1 (high mobility group 1) – a nuclear protein, released by cells in the terminal stage of apoptosis. During traumatic processes, many proteolytic and glycolytic enzymes are released in the tissues, which cleave the extracellular matrix components into heparansulfat, hyaluronic acid, the domain A fibronectin and biglican, which, in turn, activate the TLRs and induce a non-specific immune response. Now it is established that the TLRs are activated by the following endogenous ligands: 1. TLR2 - HSP60, HSP70, 96 Da glycoprotein of the endoplasmic reticulum, minimally modified LDL, HMBG1, 2. TLR3 - mRNA, 3. TLR4 - HSP60, HSP70, Gp96, fibronectin, heparan sulfate, oligosaccharides of hyaluronic acid, the protein A of surfactant, HMBG1, 4. TLR7 - mRNA, 5. TLR9 - DNA, the chromatin-IgG complexes [16].

Activation of TLRs triggers several intracellular signaling pathways. Te intracellular TIR domain (Toll/IL-1 receptor) is essential for the intracellular translation of the signal. After the detection of PAMPs, TLRs form dimmers and/or associate with other receptors or molecules that determine the interaction of the TIR domain with the intracellular adapter molecules. At the moment, scientists recognize the adapter functions of four molecules: 1. MyD88 (myeloid differentiation factor 88), 2. the inductor adapter of IFN-β containing the TIR domain (TRIF or TICAM1), 3. the adapter protein similar to MyD88 (TIRAP or MAL protein) and (4) the adapter molecule related to TRIF (TRAM or TICAM2). MyD88 and TRIF mediate two signaling pathways known as the MyD88-dependent pathway

Table 1

The Toll-like receptors and the variety of ligands recognized by them (Wikipedia)

Receptor	Ligand(s)	Ligand location	Adapter(s)	Location	Cell types
TLR 1	**Multiple triacylated lipopeptides**	Bacteria	MyD88/MAL	Cell surface	Monocytes/macrophages; a subset of dendritic cells; B lymphocytes
TLR 2	**Multiple glycolipids**	Bacteria	MyD88/MAL	Cell surface	Monocytes/macrophages myeloid dendritic cells mast cells
	Multiple lipopeptides	Bacteria			
	Multiple lipoproteins	Bacteria			
	Lipoteichoic acid	Gram-positive bacteria			
	HSP70	Host cells			
	Zymosan (Beta-glucan)	Fungi			
	Numerous others				
TLR 3	**Double-stranded RNA, poly I:C**	Viruses	TRIF	Cell compartment	Dendritic cells B lymphocytes
TLR 4	**Lipopolysaccharides**	Gram-negative bacteria	MyD88/MAL/ TRIF/TRAM	Cell surface	Monocytes/macrophages; myeloid dendritic cells; mast cells; B lymphocytes ; intestinal epithelium;
	Several heat shock proteins	Bacteria and host cells			
	Fibrinogen	Host cells			
	Heparan sulfate Fragments	Host cells			
	Hyaluronic acid fragments	Host cells			
	Nickel				
	Various opioid drugs				
TLR 5	**Flagellin**	Bacteria	MyD88	Cell surface	Monocyte/macrophages; a subset of dendritic cells; intestinal epithelium;
TLR 6	**Multiple diacyl lipopeptides**	Mycoplasma	MyD88/MAL	Cell surface	Monocytes/macrophages; mast cells; B lymphocytes;
TLR 7	**Imidazoquinoline**	Small synthetic compounds	MyD88	Cell compartment	Monocytes/macrophages; plasmacytoid dendritic cells[28]; B lymphocytes;
	Loxoribine (a guanosine analogue)				
	Bropirimine				
	Single-stranded RNA	RNA viruses			
TLR 8	**Small synthetic compounds; single-stranded RNA**		MyD88	Cell compartment	Monocytes/macrophages; a subset of dendritic cells; mast cells;
TLR 9	**Unmethylated CpG Oligodeoxynucleotide DNA**	Bacteria, DNA viruses	MyD88	Cell compartment	Monocytes/macrophages; plasmacytoid dendritic cells; B lymphocytes;
TLR 10	**Unknown**		Unknown	?	
TLR 11	**Profilin**	Toxoplasma gondii	MyD88	Cell compartment	Monocytes/macrophages; liver cells; kidney cells; urinary bladder epithelium;
TLR 12	**Profilin**	Toxoplasma gondii	MyD88		Neurons; plasmacytoid dendritic cells; conventional dendritic cells; macrophages;
TLR 13	**Bacterial ribosomal RNA sequence "CGGAAAGACC"**	Virus, bacteria	MyD88, TAK-1	Cell compartment	Monocytes/macrophages; conventional dendritic cells;

and the TRIF-dependent pathway. TIRAP and TRAM function as contact molecules with the TIRA and TRAM proteins that link MyD88 to TLR2 and TLR4 and TRIF to TLR4 [11, 20]. The adapter molecules induce successive activation cascades of different members of the kinase family, associated to the IL-1 receptor (IRAK – IL-1 receptor associated kinase).

These events end with the activation of the transcription factors NF-kB, Jun, Fos and others and the production of a

number of cytokines. An important role among cytokines, the synthesis of which is induced by the activation of TLRs, is played by the pro-inflammatory interleukins – IL-1, IL-6, IL-8, FNO-α, IL-12 and others, which regulate the systemic inflammatory response that precedes the adaptive immune responses. The set of produced cytokines is determined by the type of activated TLR. Thus, TLR4 triggers the synthesis of IL-1, IL-6 and IL-8, while TLR2 triggers the synthesis of IL-12 and TNF-α.

The synthesized cytokines induce the rapid development of the non-specific immune response and coordinate the initiation of the adaptive response through multiple mechanisms – IL-1 activates the immunocompetent cells in the inflammatory site and stimulates the production of other proinflammatory cytokines, IL-8 amplifies the neutrophils' chemotaxis, increases their activity and adhesion capacity as well as the enzyme release, IL-6 is the main regulator of the B-lymphocytes transformation into plasmocytes, IL-12 is the stimulator of NK cells, etc. In addition to inflammatory cytokines, as a result of TLRs activation by microbial products, antimicrobial effector molecules are formed, for example, nitric oxide synthases. Thus, the TLRs and the intracellular cascades, induced by them, are an integral, crucial part of the protection mechanisms of the immunological adaptation of organisms, including the human ones, coexisting with numerous microorganisms.

Stimulation of Toll-like receptors, expressed on the cell membrane, as well as in the cytoplasm and on the lysosomal membranes of dendritic cells, triggers the receptors' maturation, resulting in the synthesis of co-stimulatory molecules and in the increase of the capacity of antigen presence in these cells. Although the dendritic cells play a crucial role in the induction of the acquired immunity, the acquired immune response is initiated at the level of receptors with great phylogenetic prevalence of the innate immune system [9]. The pathogen recognition by the Toll-like receptors helps the specific targeting of the acquired immune system (involving specific B and T lymphocytes), finally leading to the elimination of the pathogen.

The complexity of the dendritic cell activation process is enhanced by the fact that there are multiple populations of dendritic cells, different by location, function and phenotype. Dendritic cells differ greatly by the types of expressed Toll-like receptors and, therefore, differ by the type of detected pathogens and the type of immune response that the receptors can initiate.

At the same time, the halting or the amplification of the TLRs activity is an important aspect of the pathogenetic mechanisms underlying many infectious or immunological diseases. Thus, it has been established that the persons with the diminished activity of the TLR4 receptors are five times more susceptible to the development of serious bacterial infections, compared to those with normal expression of the same receptor. However, the hyperexpression of TLR4 represents a favourable background for an exagerated protective reaction and the development of a severe systemic inflammatory response in people with sepsis.

After the discovery of the first Toll-like receptors in 1997, the understanding of the molecular bases of the innate immunity has been significantly improved [9]. The TLR family and their signaling pathways have been analyzed in great detail. To date, there is a significant evidence of the TLR involvement in such inflammatory and immune diseases as rheumatoid arthritis, diabetes, allergies/asthma and atherosclerosis [12]. The latest studies attest the involvement of TLRs in the diseases with an autoimmune pathogenetic element, caused by TLRs inappropriate activation by endogenous ligands, as a result, a "sterile" autoimmune inflammation is developped. The endogenous activation of TLR7 and TLR9 by certan RNA and DNA is one of the mechanisms of the lupus and psoriasis development. TLR2, TLR4 and MyD88 are the promoters of the renal transplant rejection [4]. The mutation or the deficiency of TLR4 and TLR2 affects the LDL metabolism and modifies or damages the LDL forms, which favours the emergence and the development of atherosclerosis and cardiovascular diseases [6].

The results of TLRs research have allowed the formation of a theoretical foundation and the elaboration of new pharmacotherapeutical remedies (with a low molecular mass and nucleic acid-based), whose mechanism of action is based on the specific activation or inhibition of these receptors [1, 10]. Two types of medications at the stage of clinical trials have been proposed.

1. The agonists of TLRs - apropriate for the treatment of the diseases accompanied by immunodeficiency.
2. The antagonists of TLRs - immunosuppressant remedies used in the therapy of immunoinflammatory pathologies.

The contemporary data about the nature of nonspecific immunity and the role of TLRs in its operating mechanisms allowed us to suppose that the activation of this segment of the immune system via PAMPs carrying drugs can form a rapid and non-specific defense against a large number of pathogens. Modern vaccines that are elaborated on the base of this concept would be able to induce a rapid and effective protection reaction against microorganisms that are resistant to traditional antibacterial drugs [5, 18]. On the base of the purification of the viral protein that inactivates TLRs the vaccines against the hepatitis B virus (HBV), the human papilloma virus, the herpes simplex virus, the respiratory syncytial virus and the Epstein-Barr virus have been tested.

The ongoing research in this field is expected to improve not only the vaccines against microbes, but the vaccines designed to trigger or to improve the anti-tumor immune responses as well. The progress in this area depends both on the better use of adjuvants designed to enhance the innate immunity by stimulating the Toll-like receptors and other similar receptors and the methods for the transformation of the dendritic cells into the cells that contain a sufficient amount of antigens [7].

Another type of progress concerning a clinical application is expected to be achieved in the domain of the autoimmune and the inflammatory diseases treatment. For example, the promising results have been obtained, with the help of animal

models of inflammatory diseases, both by interfering with the innate immunity through the blocking the TLRs and by inhibiting the immune response associated to the illness caused by the manipulation of dendritic cells.

The contemporary data regarding the role of Toll-receptors in the formation of the immune response, significantly facilitate the understanding of the mechanisms of the emergence of allergic, autoimmune, neoplastic, polyetiologic infectious and inflammatory endogenous conditions. These data create the theoretical foundation necessary for further elaboration of new methods and algorithms for prevention, diagnosis and treatment, based on the knowledge of the structure, properties, functions and location of TLRs [3].

In conclusion, based on the results of the studies during the last 10-12 years, we can state that the nonspecific immunological protection system is based on a limited set of specific membrane receptors, which have been called molecular 'pattern recognition receptors' (PRRs), which also include the Toll-like receptors – TLRs, that recognize pathogen-associated molecular patterns (PAMPs). The recognition of pathogens by Toll-like receptors helps the acquired immune system to find a target (involving specific B and T lymphocytes) and ultimately to eliminate the pathogen. The TLRs are involved in the pathogenesis of the inflammatory, immune, autoimmune and neoplasic diseases. The study of the TLRs has allowed the formation of a theoretical foundation and the elaboration of new pharmacotherapeutical remedies, whose mechanism of action is based on the specific activation or inhibition of the TLRs.

Thus, the scientists have accumulated not only conclusive fundamental data about the nonspecific immunity mechanisms and made attempts to practically apply the results, but they have also managed to integrate the nonspecific immunity mechanisms with those of the adaptive immunity, thus, forming a base for the substantiation of the new branch in immunology – the integral immunology, which creates the prerequisites for the inauguration of new therapeutic, diagnostic and prophylactic strategies in the future.

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