The Study of Temperature-Dependent Molecular Mechanisms of Infection Development as a Key to the Development of Modern Prophylactic Drugs (Review)

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Discoveries in molecular biology made in recent decades enabled to estimate a key role of Toll-like receptors (TLRs) in initiating innate and acquired immunity in the pathogenesis of inflammatory and autoimmune reactions. The increasing clinical significance of infections associated with gram-negative flora, and structural peculiarity of their cell membranes determined the aim of the review — synthesis of modern ideas about molecular mechanisms of infections caused by microorganisms of this type. As an actual model for the implementation of these molecular strategies the authors considered temperature-dependent immunogenicity variations of pathogenic bacteria, *Yersinia* species, depending on the number and types of acyl groups of lipid A of lipopolysaccharide of the outer membrane that has a modulating effect on the receptor TLR4 sensitivity, regulating the immune response of the body in infections caused by gram-negative bacteria. The results of numerous studies on molecular mechanisms of yersiniosis and other infections enabled to estimate that the patterns of pathogen associated molecules of gram-negative bacteria when interacting with recognizing the receptors transmit numerous signals to immune cells, and can therefore be used as a natural, innate adjuvants activating an adaptive immune response of the body. These low molecular weight natural adjuvants resembling molecular components of lipopolysaccharides are currently efficiently use to develop modern vaccines designed to activate the work of innate immune system and induce the production of inflammatory mediators.

Key words: bacteria; lipopolysaccharides; TLR4, *Yersinia spp.*; immunogenicity; vaccine development.

Advances in molecular biology at the end of the XX century essentially changed the conception of pathogenesis of infectious diseases. The revolutionary discovery and study of Toll-like receptors (TLRs) by Hoffmann (1996), Medzhitov, Janeway (1997) and Steinman (1998), and investigation of the mechanisms of their participation in activation of cell immune response contributed greatly to the understanding of the role of the innate and acquired immunity in the pathogenesis of inflammation and autoimmune diseases, and predetermined new strategies in the treatment of infections and neoplasms [1, 2], for which the authors were awarded the Nobel prize (2011). In the modern paradigm of infectious disease pathogenesis TLRs occupy the central place in the initiation of cell innate immune reactions and play a key role in early revealing of pathogens [3–5].

Each of these receptors is thought to recognize a definite molecular "image" of microorganisms for a rapid mobilization of the more ancient innate immune effector system and subsequent induction of the acquired immunity [2, 6, 7].

One of the urgent problems of modern medicine is infectious diseases caused by gram-negative bacteria. A leading etiologic role in the emergence of hospital-acquired infections belongs to them. Gram-negative

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agents are the most common cause of heavy sepsis, which is highly refractory to antibiotic therapy due to complicated mechanisms of multiple drug resistance in the causative agents of this group. One of the unique properties of gram-negative bacteria is the structure of the outer cell membrane, whose main component are lipopolysaccharides (LPS), which perform the role of endotoxins during infection development [1, 8, 9].

Pathogenic species of *Yersinia* genus — *Y. pestis* (a causative agent of the plague), *Y. pseudotuberculosis* (a causative agent of pseudotuberculosis) and *Y. enterocolitica* (an agent of intestinal yersiniosis) — are typical gram-negative bacteria, inducing infections of various severities, but using similar pathogenesis strategies. Despite the differences in the ways of spreading these infections and their severity, all these bacteria species possess similar pathogenetic mechanisms associated with functioning of chromosome genes and virulence plasmid (pCD1 in *Y. pestis* and *pYV* in enteropathogenic *Yersinia*), type III secretion system [10, 11]. The characteristic biochemical sign of the pathogenic *Yersinia* species is the availability of two isoenzymatic systems functioning independently in the external environment and in the warm-blooded organism and capable of changing depending on the specific conditions of microorganism existence [11]. These properties make it possible to consider *Y. pestis, Y. pseudotuberculosis* and *Y. enterocolitica* as facultative psychrophiles and perspective biological models for studying temperature-dependent modulation of microorganism pathogenicity, associated with molecular mechanisms of its induction.

The aim of the review is to summarize modern views on molecular mechanisms of developing infections, caused by gram-negative microorganisms, and to implement strategies on the basis of temperature-dependent variation of immunogenicity of pathogenic *Yersinia* species.

Molecular mechanisms of activating immunological protection

Maintaining homeostasis on the cellular and molecular level of organization is performed by the immune system, which protects and makes it possible for multicellular organisms to exist in the pathogenic environment. Until recently molecular mechanisms of activating immunological protection of multicellular organisms against infectious agents and their recognition remained obscure.

At the end of the XX century an important step to understanding these mechanisms was made by a discovery of the first signaling receptors, TLRs, from the family of pattern-recognizing receptors (PRRs), which induce a cascade of events, including production of anti-inflammatory chemokines and cytokines, activation of compliment, recruiting of phagocyting cells, and mobilization of professional antigen-presenting cells [12–15].

At the beginning of the XXI century TLRs group grew up to more than 10 types of receptors, which composed a numerous PRRs family. Depending on the form of existence some more groups of receptors, except for TLRs, are distinguished: secreting extracellular receptors present in the bronchoalveolar secretion as free components; a group of membrane receptors, participating in endocytosis; kinase receptors, and a group of intracellular cytosol receptors [3, 5, 16, 17].

The task of the innate immunity is to destroy pathogens penetrating into the organism using genetically coded strategy of self–nonself recognition, whereas acquired (adaptive) immunity is to produce specific antibodies on the basis of coordinated work of T- and B-lymphocytes [10, 18–20]. With better understanding of the role of the innate immunity against microorganisms (“nonself”) and of maintaining cellular homeostasis (“self”) the assessment of its connection with the phenomenon of programmed cell death (PCD) becomes significant [3, 7, 21, 22].

The innate immunity is the first line of defense of the organism against pathogenic agents [23]. It is associated with the function of signaling TLRs located on the surface of many cells of the body. The function of TLRs is to recognize a pathogen and to contact with the general conservative molecular structures of the pathogenic microorganisms, called pathogen-associated molecular pattern (PAMP) [24]. This primary recognition of pathogens is important for immediate innate immune response, production of pro-inflammatory cytokines (TNF-α, IL-6 and IL-12), induction of inflammatory reaction and bactericidal mechanisms and infection breaking, and also for subsequent triggering of specific acquired immune response [18, 24–26].

Common for the whole class of microorganisms and highly conservative PAMPs are a sort of markers of sufficiently large microorganism clusters. They play a decisive role in the pathogenesis of microbe inflammation, survival and proliferation of bacteria [27]. This makes PAMP an excellent target for immune recognition, and PRRs of the host organism cells after the recognition of bacterial viral or fungal pathogens initiate the subsequent activation of the acquired immunity: dendrite cells stimulate T-lymphocytes inducing a cascade of immune reactions and antibody synthesis, which kill bacteria and eliminate them from the body [28–30] (Figure 1).

It is important to note, that in contrast to the bacterial pathogenicity factors, molecular structures of PAMP are not unique for some microorganism species — they evolved according to their physiological functions, which are not connected with the host–pathogen interaction, and have existed long before a host organism and its cellular receptors [31]. In contrast to PAMP microorganism pathogenicity factors have developed as

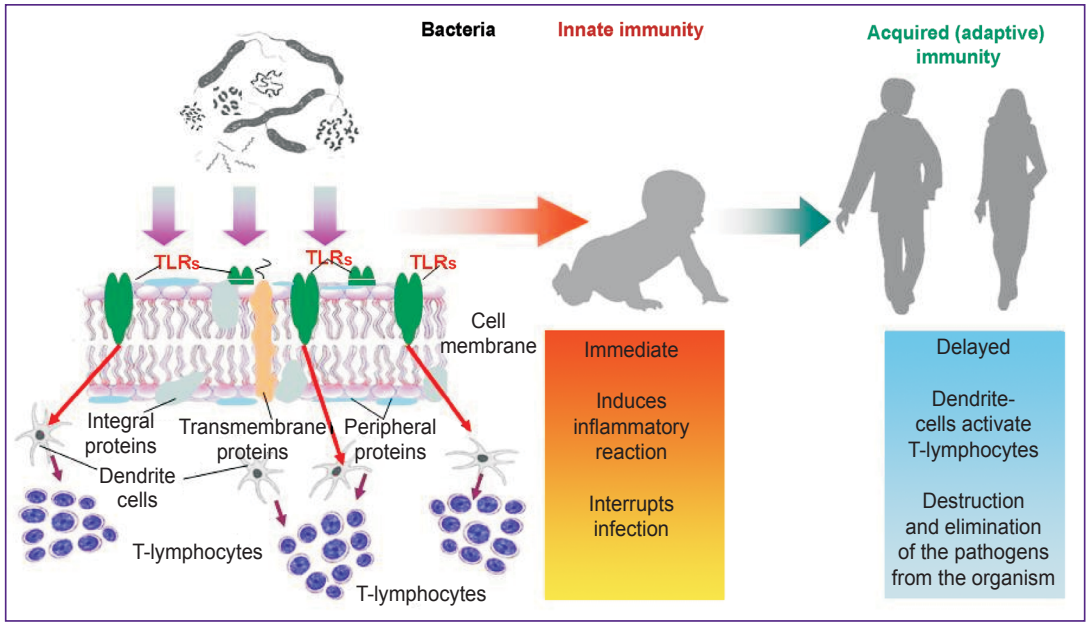

Figure 1. Modern scheme of the immune response. Invariant molecular patterns of infectious agents are recognized by signaling TLRs of the body cells, that initiates immediate innate immune response and subsequent activation of the adaptive immunity

a result of their adaptation to specific conditions of the host organism [28, 32, 33].

Thus, when infectious agents get inside the body, the primary immune response is based not on the identification of specific antigens, but rather on the recognition of definite patterns (types) of pathogen-associated molecular structures by specific PRRs of the organism cells [31, 34].

Microorganisms are known to develop various mechanisms of molecular transformation of the cell membrane main components, capable to alter immunogenicity of PAMP, which is an important strategy in the ability to avoid or modulate the immune response of the host organism [28, 32, 35, 36].

Activation of TLRs is a triggering mechanism for forming inflammasomes (specific protein complex) in different types of microorganism cells: macrophages, neutrophils, monocytes and others [9, 23, 37]. By means of inflammasome activation secretion of interleukins and interferons is realized in the cells, mechanisms of PCD (programmed cell death) are initiated (apoptosis, autolysis, pyroptosis or necrosis) resulting in intracellular degradation and bacteria death [9, 37].

So, biological expedience of forming inflammasomes and activation of PCD forms is considered as a limited death of infected cells in order to maintain the viability of multicellular organism with subsequent induction (or without it) of the cascade of inflammatory and immune reactions [31, 38, 39].

Studying the processes going on after TLRs activation revealed a close link between the innate and acquired immunity as well as PCD mechanisms, which combined all protective reactions of the body in a single system [38, 40]. The task of a grouped suppression of pathogens using a limited number of receptors was solved by the development of TLRs system, recognizing conservative molecular sequences of microorganisms absent in higher eukaryotes and being common for the main types of prokaryotes [39]. Depending on the massivity of the microbe action and pathogenicity of bacteria these signal receptors trigger the mechanisms of inflammation activation, immune response and destruction of the infected cells [39–41].

Findings of genetic investigations bear evidence of the uniqueness of PRR functions. It has been shown, that in case of insufficiently active or defective function of these receptors a proper response to a pathogen does not occur or it may take excessive forms leading to a systemic inflammatory response syndrome, septic shock and polyorganic insufficiency [32, 42]. On the contrary, in case of PRRs hyperfunction the development of chronic inflammation or autoimmune diseases is possible [32, 40, 43].

Molecular ligands of gram-negative bacteria and receptors relating to them

Currently, one of the most important fields of numerous scientific investigations is identification of molecular ligands of various microorganism categories and determination of TLRs related to them.

In medical classification of the most pathogenic bacteria for a human being two categories are significant: gram-negative and gram-positive. Each category of

Types of molecular patterns (PAMP), ligands and receptors (PRRs) of gram-negative and gram-positive bacteria [35]

Bacteria categories	Molecular patterns (PAMP)	Ligands	Receptors (TLRs)	Biological effects	References
Gr (–)	Lipopolysaccharide	Lipid A	TLR4 Receptor complex TLR4-MD-2 CD14-LBP complex: Scavenger-receptor TLP5 and TLR9	Recognition of LPS and initiation of inflammatory response Intensification of the inflammatory response initiated by TLR LPS endocytosis (noninflammatory), phagocytosis (?)	Poltorak et al., 1998 Munford, Varley, 2006 Sjölinder et al., 2008 Fedele et al., 2010 Moranta et al., 2010 Zaybert et al., 2010 Jin Lee, 2008 Matsuura, 2013
including *Yersinia*	Flagellin, CpG–DNA	Lipid A	TLR4		Dessein et al., 2009
Gr (+)	Lipoteichoic acid, lipoprotein, peptidoglycan, flagellin, CpG–DNA	Not determined	TLR2	Initiation of inflammatory response	Ru et al., 2009 Müller-Anstett et al., 2010

microorganisms is characterized by the appropriate group types of PAMP and TLR receptors corresponding to them. At present, over 10 specific receptors are known. They are located on the surface of human cells and recognize different molecular patterns [34, 36, 44]. Thus, for gram-positive bacteria and yeasts such types of PAMP are lipoteichoic acid and peptidoglycan, and receptor TLR2 is related to them; for viruses it is a double-chain RNA and a corresponding TLR3 receptor, for gram-negative bacteria — TLR4, microorganism flagella are identified by TLR5 receptors [45]. These receptors are transmembrane proteins, which are composed of an extracellular part and intracellular domain, containing Toll-interleukin-1-receptor (TIR) [18, 25, 31]. Some TLRs require supplementary protein-co-receptors for recognition and binding ligands to perform their representative functions, for example, TLR4 needs myeloid differentiation factor 2 (MD-2) for its functioning [25, 31, 45] (See the Table).

A distinctive feature of gram-negative bacteria, which are the object of the present review, is a double-layer cellular membrane. The outer layer contains highly ordered LPS molecules, being the most characterized PAMP inductor of the innate immune response of gram-negative bacteria. In the organisms bacteria LPS plays a role of endotoxin — getting into the bloodstream it may evoke a cascade of events including temperature rise, reduction of arterial pressure, sepsis, shock [12, 28, 34, 40].

Still in 30s of the XX century it was shown, that LPS represents a thermostable macromolecular complex of protein, lipid and polysaccharide [5]. Until recently it was considered, that LPS, being the main highly immunogenic component of the outer membrane, possesses a wide spectrum of biological action on the organism, triggering, as other antigens, synthesis of specific antibodies as a result of interaction with immunoglobulin receptors of B-lymphocytes [12, 28, 46, 47].

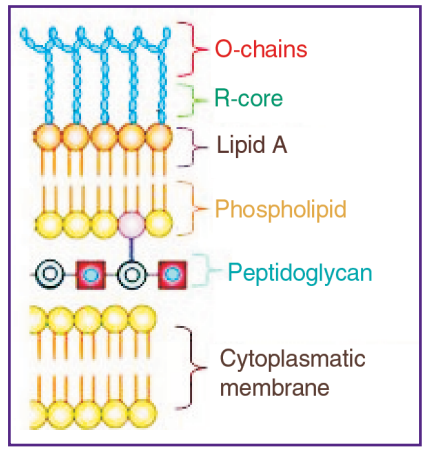

Figure 2. Schematic representation of the main structure of the double-layer membrane of gram-negative microorganisms: the outer layer contains liposaccharide molecules, consisting of lipid A, R-core and O-chains of oligosaccharides

However, the results of the investigations carried out in the 70s of the last century by Andersson et al. [48] and Weismann et al. [49], allowed them to establish, that LPS of gram-negative bacteria is a typical molecular pattern for this category, which, when getting into an organism, is first recognized by PRRs of the body as “unself”. This activates innate immune response, which is, as a rule, of the protective character. But in case of endotoxemia it may be a cause of excessive activation and impairment of inflammatory reaction regulation, inducing sepsis and disorder of organ and system functions [50, 51].

LPSs are found to be a family of related macromolecules, characterized by a common structure (Figure 2). Electron microscopy shows LPS as a fragment of a cellular membrane of diverse forms with a double-layer structure — an outer hydrophilic layer and inner hydrophobic one — lipid A [50, 52, 53]. A complete

LPS molecule is composed of a carbohydrate part, directed towards the environment and contains side polysaccharide chains (O-chains), R-core covalently bound to a lipid part, called lipid A, and an anchoring LPS in the outer membrane [54]. O-chains are characterized by an extremely high structural variability even within one bacterial species, being a chemical base for serological classification of separate genera, species and types of bacteria in accordance with their O-antigenic determinants. Great deals of native structural variants of O-specific oligosaccharides, determining antigenicity of LPS, have been described by now, as well as specificity of interaction of a bacterial cell with other biological systems, including immune system [54–56]. On the contrary, R-core and lipid A are conservative structures in a phylogenetic respect [46, 51, 57].

The architecture of hydrophobic lipid A, being the main structural component responsible for LPS toxicity, as well as its modifications have been intensively studied on *E. coli* cultures [28, 42]. Lipid A has rather a complex structure comprising fatty acids, glucosamine and the residues of phosphoric acid [42, 58]. Despite a high conservatism of this part of LPS, several types of lipid A, differing by the number of acyl groups (from 3 to 6), may compose each microorganism species. Specific structure of lipid A depends on the environmental temperature, where the microorganism live, its life cycle, and plays an important role in the strategy of gram-negative microorganism virulence and pro-inflammatory activity [12, 59, 60].

As has been already mentioned, a signaling PRRs receptor for matrix LPS molecules is TLR4 receptor, discovered in recent decades, which recognizes a general picture of an infectious agent and is expressed on the membrane of the immunocompetent cells [34, 55, 61, 62].

A chemical structure and biological effects of LPS were comprehensively studied and discussed at the end of the XX century [11, 19, 26]. This specific component of the gram-negative bacterium cell wall is released immediately after destruction of the bacterial cell or is extracted from bacterial membranes by lipopolysaccharide binding protein (LBP), circulating in blood serum and pertaining to acute-phase proteins. A generated protein-receptor complex, LPS–LBP, binds to CD14 receptor on the surface of monocytes, macrophages and granulocytes [12, 38, 45, 63], which may be in a soluble form (sCD14) or bound to a cellular membrane by a GPI-anchor. The function of CD14 consists in presentation of LPS and stimulation of the TLR4–MD-2 receptor complex by it [12, 56, 60, 63] (Figure 3).

Stimulation of TLR4 is a multistep process, during which a cascade of signaling pathways is activated. These pathways induce the production of pro-inflammatory cytokines, chemokines and adhesion molecules with the help of transcription factors NF-kB and AP-1 [63, 64–66]. In combination with LBP, CD14 and MD-2 proteins this signaling receptor alters a matrix structure of LPS, forming protein-monomer complexes LPS–LBP, LPS–CD14 and LPS–MD-2, which together with cytosol adaptor proteins (MyD88, TRIF, TRIF/TRAM) realize one of the pathways of signal transmission, activate monocytes, macrophages, and neutrophils [36, 66–68]. LPS under an electron microscope looks like a fragment of a cellular membrane of various form with a double-layer structure — an outer hydrophilic layer and inner hydrophobic one — lipid A. Albumin is a necessary factor for binding reaction [65, 67, 69–73].

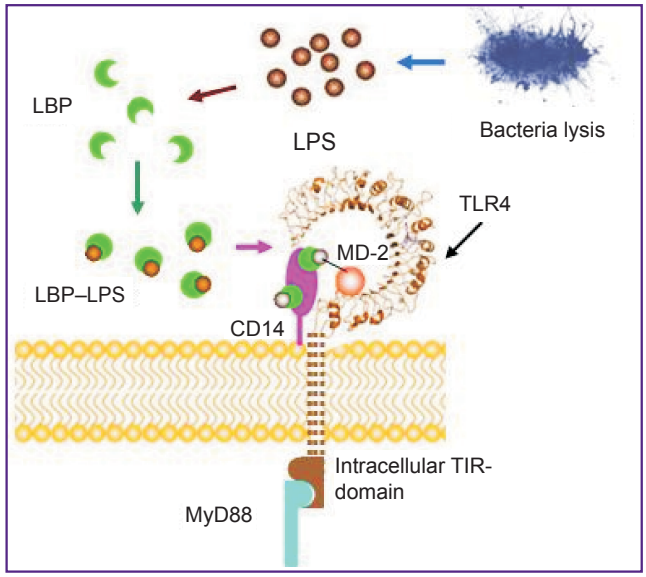

Figure 3. A simplified scheme of recognizing LPS of gram-negative bacteria by TLR4–MD-2 receptor complex of the host cell. LPS is released immediately after bacteria destruction and binds to LBP. The formed protein-receptor LPS–LBP complex binds to CD14 cluster, which presents LPS to the TLR4–MD-2 receptor complex. Then initiation of the further immune response takes place with the help of intracellular TIR-domain of the signaling receptor and My88 cytosol adapter protein

To illustrate this mechanism an example of pathogenic species of *Yersinia* genus, *Enterobacteriaceae* family, will be presented below. Of 17 species of microorganisms pertaining to this genus only three — *Y. pestis* (a causative agent of the plague), *Y. pseudotuberculosis* and *Y. enterocolitica* (agents of pseudotuberculosis and intestinal yersiniosis) — are pathogenic for a man and rodents [11, 74–77].

All pathogenic species of *Yersinia* are referred to the facultative psychrophiles, and are characterized by a wide temperature range for their growth. Despite various ways of invasion, they affect lymphoid tissues and inner organs of a host organism. A distinctive feature of yersinioses is their clinical polymorphism, different variants of the course character and duration. Pathogenicity of *Yersinia* is mainly connected with

their ability to resist the mechanisms of innate immunologic protection, such as phagocytosis, and induce the inflammatory response by macrophages and neutrophiles [77, 78, 79].

In the majority of investigations molecular mechanisms of infectious processes and a pathogenic role of LPS molecular patterns in the development of infections, caused by gram-negative agents, and their recognition by TLR4–MD-2 receptor complex, was studied on the model of lipid A of the pathogenic species of *Yersinia* genus [75, 76, 78, 80].

Signals for immunostimulating activity in the human or mammal organisms after the interaction of LPS (lipid A) with the TLR4–MD-2 receptor complex cause definite changes of the receptor. The content of extracellular TLR4 domain is not enough for LPS recognition, but presence of MD-2 in the protein complex is essential for ligand detection [81]. When lipid A is binding, MD-2 protein-receptor induces dimerization of TLR4–MD-2 by conformation of the receptor complex surface, preparing zones of interaction and binding of the ligand with a C-terminus fragment of the extracellular domain of the TLR4 molecule [12, 25, 82–86].

More than 50 years ago *Y. pestis* V-antigen (LcrV), one of the important factors of pathogenicity, coded by *lcrV* gene, was described [86, 87]. This gene is located on the pCad-dependence plasmid, present in all *Yersina* species pathogenic for a human [83, 88–90]. Production of LcrV was established to correlate with its virulence [91, 92–94]. In the works of Sing et al. [86, 95] it was shown, that LcrV is capable of modulating the immune response of the host, changing the production of IL-10 cytokines by macrophages, and also has a crucial importance for transferring Ypos effector proteins in the cytoplasm of the infected eukaryote cells.

For this purpose *Yersinia*, like other gram-negative bacteria, use type III secretion system, which works only in direct contact of bacteria with the host cells. The results of the investigations performed by Sing et al. [86] also prove the role and participation of *lcrV* gene in coding type III secretion system and direct participation of this LcrV protein product in the regulation of *YopD* secretion [86, 93]. Later these investigators showed that CD14 and TLR4 appeared to be cell receptors responsible for the production of IL-10, induced by LcrV [87, 96–98].

Researches carried out at the beginning of the XXI century showed, that alterations of lipid A acylation are critical for TLR4 activation, and variations of the acylic group quantity may modulate receptor activation [99, 100]. It has been estimated, that the reaction of human cells to the changed structures of lipid A is more expressed in relation to the number of acylic groups, compared to that of mice. For example, hexaacylated modification of lipid A in case of interaction of human and mouse macrophages with TLR4 changes the receptor condition, equally increasing the biological response. At the same time, tetraacylated modification of lipid A shows significantly weaker agonistic influence on the mice macrophages than on the human macrophages [97, 99–103].

Y. pestis is known to be one of the most dangerous bacterial pathogens, being an etiological agent of bubonic and pneumonic plague and penetrating into the human organism through the bites of fleas, infected by rodents via the contact or droplet pathways [104]. In contrast to other representatives of this family (including *Y. pseudotuberculosis* and *Y. enterocolitica*), causing intestinal infections, *Y. pestis* are not able to live and proliferate in the environment for a long time, circulating in the natural foci of the rodent-carriers and insect-transmitters (fleas) [105–107].

A remarkable property of *Y. pestis*, inherent to all *Yersinia* species, i.e. their ability to multiply at various temperatures, allows them to adapt to the changing conditions of the habitat. The environmental temperature for fleas, living in the rodent holes or mammal fur, is about 25°C, while the temperature of rodent and human body is about 37°C. Thus, in the infectious cycle of *Y. pestis* two temperature regimes are present, at which this *Yersinia* species not only survive but in various ways uses its pathogenic factor potential [108–110].

For instance, activation of such factors of *Y. pestis* virulence as I-antigen [105], pH6-antigen [107], Yops proteins [44], and type III secretion system [111, 112] occurs at 37°C, while the production of the mouse toxin [113] necessary for replication of bacteria in a flea intestine is induced at 27°C and is inhibited at 37°C [114].

A high pathogenicity of *Y. pestis* is mainly determined by the unique capability of bacteria to overcome protective mechanisms of mammals, thus providing the survival during the whole life cycle [108, 112, 113, 115]. LPS of *Yersinia* contributes much to this feature [98].

In recently published reviews by Knirel, Anisimov [115] and Matsuura [34] new data are presented on the structural peculiarity of *Y. pestis* LPS and its biological properties. The results of the investigations, carried out lately on wild mutant isogenic strains of *Y. pestis* give evidence of the fact, that LPS of these bacteria is a polyfunctional factor of pathogenicity, playing a key role in temperature-dependent adaptative strategy of this agent [34, 116].

As a majority of pathogenic microorganisms, forming rough colonies, *Y. pestis* produces R-type LPS, whose carbohydrate part does not contain polysaccharide chains, O-antigen, and is limited by a core, in contrast to LPS typical for the majority of bacteria forming smooth S-type colonies, in which polysaccharide O-antigen determinants also are present [107, 110]. This attribute of *Y. pestis* distinguishes this pathogen from other *Yersinia* species, and plays an important role in the pathogenesis of the plague, being an essential part of the strategy of overcoming by a bacterium protective mechanisms of the host at the early stages [110, 112].

But some specific features of LPS structure (including temperature-dependent structure variations both of the core and lipid A) were inherited by *Y. pestis* from *Y. pseudotuberculosis* without any noticeable changes [20, 96, 115].

Y. pestis LPS structure and its biological properties vary depending on the external conditions [115, 117–120]. Some regularity in the variations of the lipid structures in different species of pathogenic *Yersiniae* (like in other species of gram-negative bacteria) were revealed, when they were replicating in diverse ambient temperature conditions [115, 117]. The main structural and biological characteristics of *Y. pestis* LPS were studied in detail on the preparations isolated from bacteria cultured at different temperatures, which were similar to their habitat in the warm-blooded mammals (37°) and poikilothermal fleas (20–28°C) [115, 119].

The structural alterations of lipid A, making up *Y. pestis* LPS, associated with these two temperature ranges, were also found with the help of MALDI-TOF mass-spectrometric analysis [121, 122]. The authors showed, that the content of various acylated forms of lipid A depends, to a large extent, on the conditions of culturing. It was presented by different hypoacylated types of spectrum: from hexa- to three-acylated, when *Y. pestis* were cultured at 27°C, and from tetra- to three-acylated types, when bacteria were grown at 37°C [121]. Besides, LPS isolated from *Y. pestis* grown at 37°C caused a weaker immunological response of human macrophages than LPS of bacteria cultured at 27°C [122].

In the recent investigation by Kanistanon et al. [25] it has been shown, that stimulation of TLR4–MD-2 receptor complex of macrophages by lipid A at the temperature of the human body in contrast to the temperature of poikilothermal fleas caused decrease of pro-inflammatory cytokine production, including IL-12p40 and IL-6 [25]. Thus, temperature-dependent conformation of *Y. pestis* lipid A enables the pathogen to avoid immune response, associated with the reduction of ligand immunogenicity.

The mentioned structural alterations of lipid A are observed at the increase of cultivation temperature from 21–28°C to 37°C, modeling the transition from the temperature conditions in the body of poikilothermic fleas to the conditions in the body of warm-blood mammals [98, 102]. The revealed shift of the lipid A type spectrum towards hypoacylated forms is obviously connected with the necessity of immunogenicity decrease and the possibility to avoid immunologic protection of the host organism.

The *Yersinia* genus includes, apart from *Y. pestis,* two important enteropathogenic species, *Y. pseudotuberculosis* and *Y. enterocolitica*, possessing much more marked facultative psychrophilic properties — the ability to proliferate at 4°C. To analyze the structural differences of lipid A forms, yersinias were cultured at 21 and 37°C [112, 115–120]. When growing at 37°C, each of these species was found to synthesize LPS, containing mainly tetra-acylated form of lipid A, while at 21°C a relative increase of hexa-acylated forms takes place. And some differences were revealed in the number and types of acylic groups in various *Yersinia* species [112, 115, 118].

Besides, LPS-containing extractions of these *Yersinia* species, cultured at 21°C stimulated human macrophages much more intensively than similar preparations prepared from the culture of these bacteria grown at 37°C [55, 79, 93]. The findings obtained testify, that production of less immunogenic types of LPS after invading the mammal body is a conservative thermodependent strategies of *Yersinia* genus bacteria, playing an important role in their life cycle, realization of pathogenicity, and development of the infectious process [55, 79].

Thus, the ability of pathogenic *Yersinia* to temperature-dependent modulation of acylated lipid A due to their psychrophilic properties, modulates immunogenicity of *Yersinia spp*., and therefore the ability of TLR4 of the host to recognize various molecular LPS models. This is an important pathogenic feature of yersiniosis development, explaining probably clinical polymorphism, the character and duration of infections. Numerous investigations, carried out on these models during the last decades, found the complicated interrelations between the ability of the pathogenic species of *Yersinia* to modulate acylation of lipid A, and also the ability of the host organism to recognize molecular patterns and react differentially to these conformational ligand modifications [92, 102].

The revealed ability to thermodependent variation of the immunogenicity is characteristic only for pathogenic species of *Yersinia* genus. Other enteropathogenic gram-negative microorganisms of *Enterobacteriaceae* family, for example, *Escherichia spp., Salmonellas spp.* and *Shigella spp.* have LPS in the composition of molecular patterns mainly in the form of hexa-acylated lipid A, highly immunogenic for the TLR4–MD-2 human receptor complex. It speaks of the fact, that in the molecular mechanisms of infections, associated with the pathogenic *Yersinia* species, more complicated strategies of avoidance, connected with the temperature-dependent structural modifications of lipid A, are used [121–124].

An important role in the outcome of infectious diseases belongs to the production by macrophages and other cells of the immune system the key pro-inflammatory cytokines, including tumor necrosis factor α (TNF-α) — the basic mediator of septic shock (endotoxemia), developing under the action of LPS. In *Y. pestis*, like in other gram-negative bacteria including enteropathogenic species of *Yersinia* (*Y. pseudotuberculosis* and *Y. enterocolitica)*, cytokine-inducing activity of LPS is transmitted via TLR4

receptor and is determined by the structure of lipid A [122].

A limited biological activity of a high-temperature, low-acylated form of *Y. pestis* LPS may play a significant role in overcoming by bacteria protective mechanisms of warm-blooded animals. Meanwhile the system of the innate immunity is being effectively stimulated by highly acylated forms of LPS, low acylated forms are not recognized by TLR4 receptor and therefore do not activate innate immunity via TLR4–MD-2-dependent pathway. Moreover, in the experiments with macrophagal human cell lines [113, 116–118] and dendrite cells [90, 110–113] LPS from *Y. pestis* cells grown at 37°C acted as an antagonist effectively suppressing TLR-4-depended pro-inflammatory response.

However, the referred above conformational alterations of LPS did not answer the main question: how the metabolism of *Yersinia* is regulated in response to the changes of the ambient temperature, and what structures of the bacterial cell are responsible for its "measurement". The answer was received in 2009 [125].

Enteropathogenic *Yersinia*, whose life cycle is connected with circulation in nature between the objects of the environment (soil, water) and mammal organisms and is characterized by alternation of thermal living conditions, often use mechanisms of temperature probing for regulation of virulence gene expression. German scientists from the Helmholtz Center of Infection Research discovered previously unknown mechanism of temperature control by pathogenic *Yersina* species [28, 125]. Enteropathogenic *Yersinia* species were estimated to possess a tool, where RovA protein (one of the DNA-binding proteins) works as a unique thermometer.

In general terms, RovA senses the changes of the temperature directly by the alterations in the protein conformation, thus modulating it by DNA-binding capability. The same protein regulates the activity of biological bacteria functions, including pathogenicity factor, depending on the ambient temperature. RovA was found to be a multifunctional sensor protein, measuring the environmental temperature and controlling a wide spectrum of metabolic processes in the bacterial cell [28, 125, 126].

Conclusion

Innate immunity and virulence of microorganisms are extremely complicated and interdependent phenomena. The study of specific features of infectious disease pathogenesis, caused by the pathogenic species of *Yersinia*, which are connected with molecular mechanisms of pathogen liposaccharide structures, may be a key to discovering the mechanisms of innate immune defense of the body against pathogenic gram-negative bacteria. Specific differences in TLR4–MD-2–LPS interaction in *Yersinia spp.*, found in the course of experimental studies on animals, do not so far allow the researchers to extrapolate these results to a human and to connect them with clinical polymorphism and the extent of infection severity. However, like in learning any infection, understanding of pathogenetic mechanisms in yersiniosis is a critical step for developing effective prophylactic means.

The findings of numerous investigations of molecular mechanisms of developing yersinioses and other infections enabled investigators to estimate, that PAMP of gram-negative molecules transmit multiple signals to the cells and, consequently, can be used as natural adjuvants, activating adaptive immune response of the body.

The immune system frequently showed tolerance to the purified recombinant antigens used until recently for creation of vaccines. This phenomenon can be explained to some extent by the fact, that these antigens were depleted of molecular PAMP components necessary for activation of innate immunity. For this reason, subunit antigen vaccines appeared less effective in the induction of protective immune response.

To design modern vaccines it is currently rational to use agents and synthetic low molecular weight adjuvants resembling molecular LPS components — the main ligand of gram-negative bacteria designed to activate the work of the innate immune system and induce the production of inflammation mediators. Further studies of molecular mechanisms of infectious processes will allow the development and application of rational designs of effective adjuvants in making antibacterial vaccines for additional activation of the immune system, which is important in creating prophylactic preparations for children and patients with a compromised immune system, with consequent experimental testing of their biological properties.

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