Shared Pathogenesis of Infectious and Cardiovascular Diseases: 2005 View

Kuneš P., Mandák J., Lonský V., Palička V.
Department of Cardiac Surgery, University Hospital and Medical Faculty of Charles University, Hradec Králové
JInstitute of Clinical Biochemistry, University Hospital and Medical Faculty of Charles University, Hradec Králové,
Czech Republic

SUMMARY

The entry of microorganisms into the blood stream provokes a decline in the contractile function of the cardiac muscle. The lipopolysaccharide of gram-negative bacteria sets off production of pro-inflammatory cytokines including bactericidal concentrations of nitric oxide, which set up the first defense line against bacteremia. Simultaneously, however, there is an unfavorable impact on the performance of the cardiovascular system. The immediate menace resides in the incidence of septic shock, while in the long term it is suspected that chronic infectious diseases accompanied by low-grade inflammation play an active part in the initiation and progression of atherosclerosis. This hypothesis, as attractive as it may appear, has not yet been accepted unequivocally. The article offers an up-to-date review of the signaling cascades employing Toll-like receptors that permit activation by the lipopolysaccharide of the target cells. The same holds true for cellular activation by non-infectious stimuli. An emerging idea seems plausible that the same biologic events that serve to combat acute infection might in the long run be involved in the pathogenesis of atherosclerosis.

Key words: lipopolysaccharide, Toll-like receptors, atherosclerosis.

Address for correspondence:
Pavel Kuneš, MD.
Department of Cardiac Surgery of the University Hospital and Medical Faculty of Charles University
500 05 Hradec Králové, Sokolská 408
Czech Republic
E-mail: kunes.pavel@fnhk.cz
Fig. 1. Lipopolysaccharide binds to the CD14 membrane receptor in the target cells through the mediation of the LBP serum protein. CD14 together with LBP and with lipopolysaccharide form a complex with Toll-like receptors-4, to which MD2 protein is attached. The other Toll-like receptors do not need MD2 protein for the activation of intracellular signal transfer. TLR4 activates through the shared cytoplasm domain the TIR of the intracellular adaptation protein referred to as the myeloid differentiation factor 88 (MyD88). Factor MyD88 concentrates external signals transduced by Toll-like receptors and interleukin-1β and interleukin-18 receptors. The adaptation protein MyD88 binds and activates the IRAK-4 enzyme (kinase), joined by the signaling mediator TRAF-6. Cytoplasm components form their own entity, which separates from the original transmembrane complex. This is followed by phosphorylation of cytoplasm proteins TAK1 (TGF-β-activated kinase 1) and TAB2 (TAK1-binding protein 2). The next step is activation and phosphorylation of IKK kinase. The inactive form of IKK retains the NF-κB transcription factor in the cytoplasm. Activated IKK splits off the IκB-α inhibition unit. The IκB-α inhibition protein is thus separated from the NF-κB factor. The released NF-κB transcription factor passes from the cytoplasm into the cellular nucleus. There it induces synthesis of pro-inflammatory cytokines, chemokines and adhesive molecules.

Diagram drawn by Pavel Žáček, MD., Ph.D.

LIPOPOLYSACCHARIDE: SHARED MOLECULE OF GRAM-NEGATIVE BACTERIA

The innate immune system has only a surprisingly limited number of membrane receptors or receptor complexes available for the recognition of a surprisingly large number of pathogenic microorganisms – bacterial, viral and fungal – against which it launches a defensive, antigen non-specific immune response (7). The efficacy of this “immunity software” is founded on elegant simplicity. The limited number of membrane receptors called, as a group, pattern recognition receptors (PRRs), need not identify individual microorganisms. The object of recognition by PRRs is shared molecular structures of pathogenic microorganisms referred to as pathogen-associated molecular patterns (PAMPs). An example of one such shared molecular structure, or PAMP, is the lipopolysaccharide of gram-negative bacteria. Mechanisms of the innate immune system, specifically PRRs, do not discriminate whether the lipopolysaccharide whose presence they have just detected belongs to Escherichia coli, Salmonella typhi or to a totally different type of gram-negative bacteria. Such discrete tuning of the defensive reaction is the domain of the mechanisms of the adaptive, antigen specific immune response.

TOLL-LIKE RECEPTORS: DETECTION OF IMMINENT DANGER

The common imperative of defense reactions is the early recognition of imminent danger. This danger (danger signal) is represented at the general level by PAMPs molecules and is registered by PRRs membrane receptors. Typical representatives of PRRs are the already mentioned Toll-like receptors (8). TLR4 recognizes the lipopolysaccharide of gram-negative bacteria; TLR2 recognizes peptidoglykan and/or the lipoteichoic acid of gram-positive bacteria (9). Toll-like receptors are not, at the same time, restricted in any way to identify molecules shared by pathogenic microorganisms. PAMPs of an endogenous origin are themselves a danger signal. An interesting example is the heat shock protein or stress protein HSP60 (10). The status of this stress protein as a molecule signaling imminent danger to the organism is unique in many aspects. HSP60 of an endogenous nature is released from autogenous necrotic cells. This is highly homologous with HSP60 of exogenous origin, found in the membranes of many pathogenic or conditionally pathogenic microorganisms. Chlamydia pneumoniae, Helicobacter pylori or Porphyromonas gingivalis carry in their cellular walls a mutually close bacterial HSP60. All the above-listed microbial agents are suspected of participating in the pathogenesis of atherosclerosis that is mediated by their stress proteins HSP60. Both bacterial HSP60 and human HSP60 are identically recognized by Toll-like receptor-4 (11). To a certain degree, Toll-like receptor-2 also takes part in this process (12). Irrespective of its origin – endogenous versus exogenous – HSP60 utilizes, through the mediation of TLR4, the same signaling cascades activating an innate immune response as does lipopolysaccharide. Purely endogenous agents taking part through TLR4 in the activation of the innate immune response, contributing simultaneously to the pathogenesis of atherosclerosis, are fibrinogen and fibropectin (13, 14).

From the point of view of cardiovascular pathology, the above-mentioned leads to the following findings:

- TLR2 and TLR4 are expressed by cardiac muscle cells (15, 16).
- Their activation is linked to the progression of atherosclerosis (17, 18).
- Their activation is induced both by pathogenic microorganisms, and by endogenous agents, especially heat shock protein HSP60, and, besides fibrinogen and fibropectin, also especially by oxidized forms of low-density lipoproteins (oxLDL) (19).
TOLL-LIKE RECEPTORS AND GENERALIZED INFLAMMATORY REACTION

At the level of the whole organism, Toll-like receptors are activated in severely traumatized patients and in patients in septic shock (20, 21). Their activation also occurs in patients after open-heart surgery. In the immediate post-operative period, this group of patients manifests stronger or weaker symptoms of multiple organ dysfunction. These may develop without concomitant infectious complications (22). Such status is referred to as the “sepsis-like syndrome”. It is induced by contact activation of the blood flowing through the heart-lung machine, by myocardial ischemia and reperfusion during surgical handling of this organ, and by ischemia of the splanchic bed and passage of endotoxin or directly of intestinal microbial flora into system circulation (23). The individual genetic disposition of each individual (24), and the effect of controllable factors like cardiac output, oxygenation of arterial blood during artificial pulmonary ventilation and especially during spontaneous breathing, perioperative and postoperative blood loss, stability or instability of blood pressure and the quality of other organ functions, decide whether the systemic inflammatory response syndrome (SIRS) remains restricted to laboratory deviations only, or whether it acquires any of the unfavorable clinical forms (25). Once clinical manifestations of multi-organ failure appear, the first and most frequently affected organs are the heart and lungs (26, 27). In principle, it makes no difference whether multi-organ failure has been induced by an infectious or non-infectious complication (28). An infectious complication, however, often changes the multiple organ dysfunction syndrome into the multiple organ failure syndrome (29).

TOLL-LIKE RECEPTORS IN THE PATHOGENESIS OF ATHEROSCLEROSIS

A new finding has been included into the spectrum of lipopolysaccharide effects on the cardiovascular system: TLR2 and TLR4 induce the activation of intracellular signaling pathways, converging into the process of translocation of the transcription factor NF-κB from the cytoplasm into the cellular nucleus. These activated kinase 1β mediated, RR pressure, PHurrblet, P Pressttoon, HHuurrrbbuurrtt, PP, JJaanneewwaayy, CC AA JJrr:: ZZhaanngg, GG, Gghhoosshh, SS:: B- nuclear factor kappa B kinase activation:

TLR4 induce the activation of intracellular signaling pathways, lipopolysaccharide effects on the cardiovascular system: TLR2 and syndrome (29).

Abbreviations
CD14 - membrane receptor for polysaccharide
HSP60 - heat shock protein
IKK - IκB kinase
IRAK-4 - IL-1-activated kinase
LBP - lipopolysaccharide-binding protein
LPS - lipopolysaccharide
NF-κB - nuclear factor kappa B
oxLDL - oxidized form of low-density lipoprotein
PAMPS - pathogen-associated molecular patterns
PRRs - pattern recognition receptors
SIRS - systemic inflammatory response syndrome
TAB2 - TAK1-binding protein 2
TAK1 - TGF-β-activated kinase 1
TIR - Toll-like/interleukin-1 receptor
TLR - Toll-like receptor
TLR4 - human Toll-like receptor 4
TRAF-6 - TNF receptor-associated factor

REFERENCES
12. Vabulas, R. M., Ahmad-Nejad, P., da Costa, C., et al.: Endocyted HSP60s use Toll-like receptor-2 (TLR2) and TLR4 to activate the


Supported by Teaching Hospital Research Project No. MZO 00179906

Translation: Nada Abdallaová
On P. Kuneš: “Shared Pathogenesis of Infectious and Cardiovascular Diseases: 2005 View”

The discovery of toll-like receptors (TLR) in 1997 heralded the important progress that has been made in the understanding of mechanisms activating innate immunity to infectious diseases. It was presumed until recently that innate immunity recognizes the infectious agent on the basis of very unspecific reactions. It has become known, however, that innate immune cells (as well as other cells that are not part of the immune system) are equipped for the rapid initiation of an acute inflammatory reaction with relatively specific receptors. The natural ligand is known in seven of the ten TLRs. Two receptors have been studied more than others so far: TLR2 and TLR4. TLR4 is activated by the lipopolysaccharide (LPS) of Gram-negative bacteria, but it can be activated for example also by the respiratory-syncytial virus (RSV). In the case of TLR2, a broader range of activation ligands is known, including LPS of leptospira, peptidoglycan of Gram-positive bacteria, components of the cellular walls of mycobacteria, mycoplasma, parasites (Trypanosoma cruzi) and fungi. TLR1 is capable of distinguishing between bacterial lipopolysaccharides and lipoteichoic acid, but it is presumed that a synergy is necessary between this receptor and TLR2. One of the surprising findings was that TLR3 is activated by viral RNA. TLR5 activation is caused, on the other hand, by the flagellin of both Gram-positive and Gram-negative bacteria. It is interesting that in intestinal cells this receptor is located on the basal wall, and its activation probably occurs only after the invasion of pathogenic bacteria through the intestinal epithelium. A lot of attention in infectious diseases has also focused on TLR9, which is activated by so-called non-methylated CpG motifs (cytosin bound to guanine by a phosphate bond). CpG motives are characteristic of bacterial DNA; in human cells these nucleotides are usually methylated.

Due to the importance of innate immune response, the role of these receptors in the pathogenesis of a number of infectious diseases has been the subject of research. It can be stated, generally, that the pathogenesis of infectious diseases is affected both by excessive and by insufficient immune response. Excessive immune reaction is an important factor that may, for instance, affect significantly the clinical course and prognosis of sepsis. The role of TLR in fulminant meningococcaemia has been observed in this context. An essential pathogenetic effect of natural immune mechanisms is presumed here due to the rapid course of the disease. Despite the original presumption that TLR4 blockade could have a protective role in endotoxaemia, it seems that the opposite is true in the case of fulminant meningococcaemia. In individuals with Asp299Gly polymorphism of TLR4 a higher risk of meningococcal infection has been recorded. This has been confirmed in patients with Gram-negative sepsis of other than meningococcal aetiology. Similarly, TLR2 polymorphism is associated with increased incidence of sepsis caused by Gram-positive bacteria and TLR5 polymorphism with enhanced susceptibility to Legionnaires’ disease. In spite of these findings, in the case of most TLRs we do not yet know the significance of their activation in the pathogenesis of infection. It is not known, for instance, whether TLR activation during an RS viral infection or TLR2 stimulation by mycoplasma F protein supports the survival of these pathogens in the organism or whether it helps their liquidation.

The importance of TLR has also been studied in the context of development of new vaccines. The adjuvants that are still being used have been shown to activate various TLRs. An example is Freud’s adjuvant, containing components of mycobacterial cellular walls, which activate TLR1, 2 and 4. TLR activation is also of crucial importance for the stimulation of an adaptive (specific) immune response. As early as 4 hours after TLR activation, numerous cytokines and costimulatory molecules are produced on the antigen-presenting cells, which assist the development of an adaptive immune response. Considerable attention has been devoted recently to CpG nucleotides, which have significant immunostimulatory properties – they stimulate B lymphocyte proliferation and cytokine production by dendritic cells. These properties are very interesting from the point of view of developing new vaccines and immunostimulatory therapies.

The complexity of the role of TLRs in the pathogenesis of infectious diseases is reflected in diverse distribution of the individual receptors on the immune and non-immune cells. It appears that this distribution has a significant impact on the function of individual TLRs. For example, the TLR blockade on monocytes, which is the cause of greater susceptibility to sepsis triggered by various Gram-negative pathogens, has a preventive effect in the airways in LPS-induced asthma. Similarly, TLR4 stimulation in mice myocytes with the help of LPS has no cardiodepressive effect if this receptor is blocked. The cardiodepressive effect of LPS is achieved during simultaneous TLR4 stimulation on myocytes and monocytes or, in the case of TLR4 stimulation, only on monocytes. The currently prevailing opinion is that the cardiodepressive effect of sepsis is mediated especially by TNF-α, produced by activated monocytes and neutrophils.

A role in the pathogenesis of infectious diseases is also played by TLR activated intracellular signaling cascades, which lead eventually to NF-κB activation, to the production of both pro- and anti-inflammatory cytokines and to increased expression of adhesive and costimulatory molecules. So far the IRAK-4 defect has been described as clinically significant; the defect leads to a blockade of TNF-α, IL-1β, IL-12p40 and IL-6 production. Although these pro-inflammatory cytokines are very important in the initiation of an immune response, surprisingly, the carriers of this defect have greater susceptibility only to a very limited spectrum of bacterial infections. This is undoubtedly a demonstration of the unique ability of the immune system to substitute a defective function.

Although TLRs are extremely interesting from the point of view of pathogenesis of infectious diseases and their possible treatment, the whole range of their important functions, mutual bonds and relation to other cells is not yet known. An interesting prospect is the blockade of terminal signaling molecules – NF-κB, JNK and p38 – activating genes for a whole number of immune mediators whose dysregulation plays a role in important infectious diseases – e. g. sepsis. Since intracellular cascades are partly responsible for streamlining the activation signal (e. g. ILF-3 activation leads to the production of interferons-α and β), they could represent an ideal target for specific immune stimulation. The road leading to such output, however, is still rather long.

Translation: Nada Abdallaová

Address for correspondence:
Michal Holub, MD., Ph.D.
IIIrd Department of Infectious and Tropical Diseases of the 1st Medical Faculty of Charles University and Na Bulovce Hospital and Polyclinic
180 01 Prague 8, Budínova 2, Czech Republic
E-mail: michal.holub@lf1.cuni.cz