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The role of Toll-like receptors in viral infections – selected data

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Abstract

Toll-like receptors (TLRs) are very well known for their antibacterial, antiviral and antiparasite importance. Nowadays, there are 18 TLRs – TLR 1-13 in human, and TLR14, 15, 21, 22, 23 among others vertebrates and invertebrates. As pivotal pathogen recognition receptors (PRR) they tend to be a part of many infections. Their role in bacterial infection is unquestioned, nevertheless, this paper shows the importance of those receptors in viral infections. Virus recognition by TLRs is based on the characteristics of PAMP – dsRNA, CpG motif of viral DNA, ssRNA and viral glycoprotein envelopes.

Moreover, the role of TLRs in viral haemorrhagic disease in rabbits as an interesting matter for authors, has been described.

Key words: *Toll-like receptors, viral recognition, rabbit haemorrhagic disease.*

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Introduction

Since the discovery of Toll-like receptors (TLRs), the number of studies on their importance and possible applications has been growing geometrically. The receptors, owing to their capacity to recognise pathogen associated molecular patterns (PAMP), are a very important example of pathogen recognition receptors (PRR) and the most important receptors that condition functioning of natural immunity mechanisms – the strongest element of anti-contagious immunity, including anti-viral immunity [1-8]. So far, it has been evidenced that they are present in many cells, including immune system cells, namely lymphocytes, neutrophils, dendritic cells, mastocytes, monocytes and macrophages, as well as in epithelial cells of the digestive system and respiratory system, endothelium of blood vessels, skin, adipocytes, cardiomyocytes, fibroblasts, and many cells of other organs in mammals [1-9]. Owing to such location, they have a unique capacity of binding to PAMPs, both of bacterial and viral origin, as well as of parasite origin [1-7]. Furthermore, due to their conservative structure and location, they have an important role of "superactivators" in immunity of vertebrates, including mammals, forming the basis of their protection against microorganisms and parasites [1-7].

So far, in mammals, including humans, 13 TLR markers have been described, yet currently also the following receptors have been described: TLR14, TLR15 and TLR21, 22 and 23 [10, 11], whereas receptor TLR14 was recorded only in frogs and fish, and despite the fact that its function has not been fully recognised [10, 11], it was evidenced that in fish *Paralichthys olivaceus*, it participates in bacterial infection with *Edwardsiella tarda* [11]. In turn, TLR15, which is molecularly the furthest from all other markers from the TLR family, was observed in chickens, including in the case of infection with *Salmonella enterica* [12]. TLR21, 22 and 23 were described and recorded in some species of fish, frogs, but also in chickens [10]. It is worth stating, that until today, ligands of TLR14 have not been identified, while for TLR15 there are evidences that it recognizes unique, non-secreted, heat stable component of both – gram positive and gram negative bacteria of avian specific pathogens and *Salmonella* [13]. For the so-called 'fish-specific' TLR21, 22 and 23, the only known ligand is dsRNA and polyI:C for TLR22 [14], whereas the chicken TLR21 was shown to recognize CpG DNA like the mammalian TLR9 [15].

It is worth stating that the 13 TLR markers described so far were grouped into five sub-families: TLR2 (TLR1,

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TLR2, TLR6 or TLR1 and 2, as well as TLR2 and 6), TLR3, TLR4, TLR5 and TLR9 (TLR7, TLR8, TLR9) [1-8], while 18 TLRs currently described in vertebrates (1-15 and 21-23) [10], on the basis of phylogenetic studies, were grouped in six sub-families: TLR1, TLR3, TLR4, TLR5, TLR7 and TLR11, whereas the sub-family of TLR1 includes TLR1 described in all vertebrates, and TLR2 that is present in mammals, fish, and some birds, as well as TLR6 and TLR10 present in mammals – markers which are molecularly very close to TLR1, and TLR14 that was identified in *Xenopus* frogs and *Tetraodon* and *Fugu* fish, as well as TLR15 recorded in chickens [10]. TLR3 sub-family comprises a group of receptors that is very homogenous, and only gathers TLR3 in various species of mammals, including humans and invertebrates. In the case of two further sub-families, namely of receptors TLR4 and TLR5, these include, respectively, TLR4 and TLR5 recorded in vertebrates [10]. The next sub-family is formed by TLR7, and gathers receptors TLR7, TLR8 and TLR9, identified both in vertebrates and invertebrates. The last, sixth sub-family, TLR11, is formed by receptors TLR11, TLR12, TLR13 in mammals (mice, rats) and TLR21 detected in *Takifugu rubripes* fish, *Xenopus* frog and chickens, as well as TLR22 and TLR23 recorded in various fish species [10].

Virus recognition by Toll-like receptors

Specific and appropriately quick recognition of pathogens, including viral pathogens, is undoubtedly important for the immune system to take the necessary steps in order to efficiently fight such infections. It is adopted that PRRs, including TLRs, are of key importance during activation and support of natural immunity mechanisms [1-8]. Although most reports refer to their role in bacterial infections, without depreciating this role, it must be stated that the receptors are also of high significance when fighting viral infections [16-31]. Within viruses, four types of PAMPs must be mentioned to which TLRs are sensitive, namely: dsRNA, CpG DNA, ssRNA and envelope glycoproteins [16-31].

In the case of **dsRNA** recognition, it was evidenced that the most important receptor is TLR3, which not only allows for capturing the viral dsRNA itself, but also for its recognition in neighbouring cells, which is mediated via TIR-domain-containing adapter-inducing interferon-β (TRIF) that, by inducing phosphorylation of interferon responsive factor (IRF3) leads to production of IFN-β [16, 29, 30]. It was determined [16] that the activation level of TLR3, due to the presence of viral dsRNA and infection-related progressing secretion of IFN-α, IFN-β and pro-inflammatory cytokines, is much lower than in the case of other TLRs, and such a condition is of key importance when preventing viral infections [19]. It is known that TLR3, apart from recognition of dsRNA of viruses from the *Birnaviridae* and *Reoviridae* families, also binds to selected DNA viruses, e.g. from the *Herpesviridae* family, which was recorded during infection with cytomegalovirus in mice [6, 16, 30]. Furthermore, the receptor takes part in infections with ssRNA viruses from such families as *Paramyxoviridae*, e.g. respiratory syncytial virus (RSV), *Picornaviridae* e.g. encephalomyocarditis virus (EMCV), *Flaviviridae* e.g. West Nile virus (WNV) [19] and *Bunyaviridae* e.g. Punta Toro virus (PTV) [28]. It is also known that the receptor can recognise viral dsRNA formed during their replication, as experimentally confirmed in reference to cell infection with human viruses from the *Retroviridae* family, e.g. HIV [19, 27].

In turn, the recognition of **CpG** motif of viral **DNA** is performed in the largest spectrum by TLR9, although in the early phase of studies on this receptor its close relation was stressed only with the non-methylated CpG DNA in bacteria. It is nowadays known [16, 28, 30] that TLR9 recognises CpG DNA of viruses from the *Herpesviridae* family, such as cytomegalovirus in mice, and HSV-1, HSV-2 (*Herpes simplex* virus 1, 2), as well as certain viruses from the *Poxviridae* family (e.g. variola virus), *Adenoviridae* (e.g. human adenovirus C) and *Anelloviridae* (e.g. Torquetenovirus (TTV)). The viruses [16] the genome of which is rich with CpG DNA, via TLR9, activate pro-inflammatory cytokines and IFN-α on the MyD88-dependent pathway. It was also evidenced [16] that TLR9 activation can be inhibited by chloroquine, which prevents endosome acidification [16]. It was determined, that during virus recognition by TLR9, DC cells, macrophages and B cells are activated, and response mediated with Th1 lymphocytes is stimulated [19]. It was also stated that the appropriate ligand recognition by TLRs is related to factors that specifically "present" the ligand to the receptor [22], and this leads to specific and quick commencement of the interaction between TLRs and ligand. In the case of TLR9 [22], it was determined that for proper recognition of viral motif CpG DNA, the particle first binds to granulin – multifunctional protein rich with cysteine, produced by many cells of mammal organisms, due to which the complex is more specifically recognised by TLR9. A factor with similar function for the receptor (TLR9), can be protein HMGB1 (high mobility group box), namely chromatin-related protein involved in the process of "rendering the viral DNA visible" to TLR9s [22, 25].

In the case of **ssRNA** viruses, it was determined that their recognition is mostly due to TLR7 and TLR8 receptors [6, 8, 16], which were originally believed to recognise exclusively the synthetic derivatives of nucleic acids, such as imiquimod and resiquimod, and guanin derivatives with antiviral and anticancer properties [19, 21]. The receptors, similarly as TLR9, in order to function correctly, require endosome acidification, owing to which IFN-α is produced on a MyD88-dependent pathway [16, 29]. The role of TLR7 and TLR8 was confirmed in the case of ssRNA viruses from the *Orthopoxviridae* family, e.g. influenza virus type A, *Rhabdoviridae*, e.g. vesicular stomatitis virus (VSV), as

well as *Picornaviridae*, e.g. Coxsackie virus B (CVB) [6, 16-18, 28, 30]. Furthermore, TLR7 and TLR8 recognise dsRNA viruses from the *Birnaviridae* and *Reoviridae* families, as well as ssRNA viruses using reverse transcriptase – e.g. human immunodeficiency virus (HIV) from the *Retroviridae* family. It was also evidenced [19] that TLR7 can recognise synthetic poly(U) RNA tails. It was determined that the high level of TLR7 expression on plasmacytoid DC cells (pDC) allows for production of high volumes of IFN-α after viral infection, which makes pro-inflammatory cytokine production by the cells entirely dependent on TLR7, and it is also suspected that the receptor serves as a "sensor" of infection with many ssRNA viruses [19]. This is because RNA virus recognition with TLR7 is independent on replication, as after entering endolysosomes, the viruses are recognised by the receptor and the process of their destruction begins [19]. Moreover, in the infection with Coxsackie B virus from the *Picornaviridae* family, it was determined [28] that its detection by TLR7 is activated by FcR, owing to which the process of binding to antibodies is more effective. It was also evidenced [20] that in endosomes of murine regulatory lymphocytes CD4+CD25+, the level of TLR7 is three times higher as compared to naïve effector lymphocytes, which points to the involvement of such cells in viral infection recognition. Such observations prove [21] that the action of ligands for TLR7 and TLR8 can have modulating effect on the course of the infection with RNA viruses, related to the development of cellular response modulated with Treg lymphocytes.

In turn, recognition of viral **glycoprotein envelopes** by TLR seems to be a different process, as viruses are recognised in this way at an early phase of the infection by TLRs present on the surface of the cells, contrary to viruses recognised inside the cell at the phase of replication [16]. The mechanism of this recognition is based on the protein-protein interaction between the certain TLR and the viral envelope protein. In this case it was determined that TLR4 is activated in the case of ssRNA viruses from the *Paramyxoviridae* family, e.g. respiratory syncytial virus (RSV) – by protein F, and in the case of ssRNA viruses using reverse transcriptase – e.g. mouse mammary tumour virus (MMTV) from the *Retroviridae* family – by protein Env [6, 16]. Due to the fact that cooperation is also known between TLR2 and TLR4, it was evidenced [16, 18, 24] that the earlier (TLR2) participates in recognition of ssRNA viruses from the Paramyxoviridae family, such as measles virus, but also of DNA viruses from the *Herpesviridae* family, such as human cytomegalovirus, or herpes simplex-1. It was proven that TLR2, acting in cooperation with TLR6, can contribute to recognition of viruses from the *Herpesviridae* family, e.g. Epstein-Barr virus, as well as dsDNA virus using reverse transcriptase from the *Hepadnaviridae* family, namely hepatitis B, and ssRNA viruses from the *Flaviviridae* family, e.g. hepatitis C, and from the *Arenaviridae* family, e.g. virus causing lymphocytic choriomeningitis [26, 28, 30]. The study also evidenced that for these viruses, the particle responsible for "improving" ligand recognition is the cluster of differentiation CD14.

Furthermore, it is worth stressing again that TLR2 and TLR4 are extracellular receptors, so their recognition of viruses is not very specific, while TLR3 and TLR7, 8, 9 are intracellular receptors that are strictly in charge of recognition of viruses – intracellular "parasites". The latter (TLR3, 7, 8, 9) bind their ligands in the mature endolysosome, namely where in physiological conditions host's nucleic acids are not present, hence there should be no interference with viral nucleic acids, and due to which their fighting should not be rendered difficult [20, 21, 24, 25].

To conclude, it can be stated that the discovery of TLRs allows for explaining the mechanisms governing recognition of viruses, although not only viruses, by the host's immune system. Moreover, TLRs constitute as if a "bridge" between the elements of natural and acquired immunity, as they are factors promoting maturation of dendritic cells, and also activate acquired response through their expression also on B and T cells that condition this type of immunity [31]. It was determined [31] that activation of memory T cells by TLRs causes their powerful proliferation, but also secretion of antibodies by B cells. Furthermore, TLRs modulate the expression of regulatory lymphocytes (Treg) – the element linking natural and acquired immunity, while TLR4, 5, 7 and 8 are selectively expressed by Tregs, what caused even 10-fold increase in their suppressor efficiency [21, 25, 31].

Toll-like receptors in rabbit infection with RHDV

Rabbit haemorrhagic disease (RHD) virus from the *Caliciviridae* family is causing rabbit plague – a disease that affects both wild and farm rabbits [32-35]. Rabbit haemorrhagic disease virus is non-enveloped, with the size of 28-40 nm, density of 1.310-1.365 g/cm^3, and cubic symmetry. Inside the capsid in the form of regular icosahedron with thirty-two capsomeres, there is a single-stranded, linear, positively polarised RNA comprising 7437 nucleotides [32-35]. Despite the studies pointing to complexity of infections with viruses from the *Caliciviridae* family [36, 37] and the need for more thorough recognition of the very course of the viral infection, so far there has been just one report [38] on TLRs in rabbits infected with RHDV. The researchers [38] state that in rabbits, there is no TLR7 and TLR8, the presence of which should be obvious due to the fact that these receptors are considered as fundamental for recognition of ssRNA viruses, to which RHDV belongs. The authors, therefore, suggest [38] that the only receptor that can participate in antiviral immunity in rabbits is TLR3, although the data are still not fully confirmed. However, according to the authors of the present study, it is more probable that rabbits feature TLR2 and TLR4, and perhaps even TLR6, as these receptors have been identified as being of

key importance for viral infections related to viral infections of the liver caused by hepatitis B virus from the *Hepadnaviridae* family and hepatitis C virus from the *Flaviviridae* family, and as evidenced, and which is unquestioned, after infecting rabbits, RHDV causes relatively the greatest lesions in the liver – the main place of its replication. At present (unpublished data), studies are carried out with the objective to confirm the presence of TLR2 and TLR4, and point to the fact that the expression of TLR2 and TLR4 is inhibited by "some other marker" – perhaps TLR6 or TLR10, which belong to the same sub-family.

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