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Recognition of viral single-stranded RNA by Toll-like receptors ☆

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Abstract

The Toll-like receptors (TLR), mediating innate immune activation upon recognition of viral nucleic acids, represent promising targets for the development of adjuvants. Therefore, there is great interest in unraveling the underlying mechanisms of ligand recognition. Studies aiming to identify which sequences, nucleic acid modifications and molecular moieties of viral nucleic acids trigger or inhibit TLR activation have allowed insights into this subject, yet there are still many aspects of innate recognition of viral nucleic acids which are only partially understood. This review discusses our current understanding of TLR-mediated recognition of viral single-stranded RNA (ssRNA) by TLR7 and TLR8. Oligoribonucleotides (ORN) and small immune response modifiers such as imidazoquinolines with agonist function have served as tools to study ligand recognition. In addition, there is increasing evidence that TLR-mediated recognition of mammalian ssRNA triggers innate immune activation and plays a role in autoimmunity. Thus the development of suitable TLR7 and TLR8 antagonists could pave the way for therapeutic intervention of particular autoimmune diseases.

Keywords: Adjuvant; Dendritic cells; Immune response modifiers; Innate immunity; Pathogen-associated molecular patterns; Pattern recognition receptors; Toll-like receptors; Virus

Contents

Abbreviations: DC, dendritic cells; DAI, DNA-dependent activator of IFN-regulatory factors; dsRNA, double-stranded RNA; MDA5, melanoma differentiation-associated gene 5; PAMP, pathogen-associated molecular pattern; PDC, plasmacytoid DC; IFN-I, type I interferon; PRR, pattern recognition receptor; RIG-I, retinoic acid inducible gene I; ssRNA, single-stranded RNA; TLR, Toll-like receptor.

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1. Introduction

The pattern recognition hypothesis postulates that cells of the innate immune system possess pattern recognition receptors (PRR) that recognize pathogen-associated molecular patterns (PAMPs), which are indispensable for the life cycle of these microbes and fundamentally different from the molecular structures of the host [1]. While bacteria and protozoa indeed create glycoproteins, lipidoglycans and carbohydrate structures not found in their mammalian hosts, viruses hijack the metabolism of the host cell they have infected to replicate. Consequently, viral protein, lipid and carbohydrate structures are very similar to host molecular structures. Nevertheless, viral PAMPs belong to the most potent stimuli triggering innate immune activation with viral nucleic acids serving as the molecular structures recognized. There are three classes of viral nucleic acids namely single-stranded RNA (ssRNA), double-stranded RNA (dsRNA) and DNA, which are recognized by separate innate PRR [2,3].

Viral nucleic acids produced upon replication in infected cells are sensed by ubiquitously expressed cytoplasmic PRR such as retinoic acid inducible gene I (RIG-I), melanoma differentiation-associated gene 5 (MDA5) and DNA-dependent activator of IFN-regulatory factors (DAI) which are specific for viral ssRNA, dsRNA and DNA, respectively [4–7]. Recognition of viral nucleic acids in the cytoplasm of infected cells via these cytoplasmic PRR triggers an anti-viral response that culminates in the production of IFN-β, which confers virus resistance to neighboring cells, and in the apoptosis of the infected cell if unhindered by viral interference with these pathways [8]. In contrast to these ubiquitously expressed cytoplasmic PRR, expression of the TLRs mediating recognition of viral nucleic acids is restricted to specialized cell types. Ligand recognition by these specialized cell types such as dendritic cells (DC), NK cells, T cells and B cells is crucial for the induction of immunity in response to virus infection [9–11]. As for the cytoplasmic PRR there are specific receptors for all three classes of viral nucleic acids with TLR7 and TLR8 recognizing viral ssRNA, TLR3 sensing viral dsRNA and TLR9 being triggered by viral DNA [12–15]. These TLR-specific viral nucleic acids form a subgroup among the family of TLRs located in a specialized endosomal compartment rather than at the cell surface. The basic principles of ligand recognition by these intracellular TLRs is primarily studied by employing synthetic oligonucleotides, which are easy to manipulate, but which may not resemble the natural ligands in all physiological aspects of ligand recognition. Studying the underlying molecular principles of viral ssRNA recognition is complicated by the fact that there are two ssRNA-sensing TLRs with different but partially overlapping specificities. In human cells, ssRNA recognition by TLR7 and TLR8 has been investigated, but due to the lack of genetic knock-out approaches in the human system it is difficult to definitively separate TLR7-mediated activation from TLR8 stimulation and vice versa. Similarly, the study of TLR7 and TLR8 transfectants has only limited significance and can fail to mirror results obtained with primary TLR7 expressing cells [13]. While mouse TLR7 has been studied intensively, mouse TLR8 was thought to be non-functional and therefore, exploring TLR8-mediated recognition was largely neglected [16]. Recently, however, it was reported that mouse TLR8 is activated under specific conditions which will hopefully open up new routes of investigation [17,18]. This review will discuss the localization of TLR-mediated ssRNA recognition, cell-type specific activation via TLR7 and TLR8 and how the various agonists and antagonists that have been identified for these two PRR have shaped our understanding of their specificity and of their role in anti-viral defense and autoimmunity.

2. Location of ligand recognition by TLR7 and TLR8

The TLRs specific for nucleic acids are localized intracellularly sampling the content of a specialized endosomal compartment, which requires endosomal maturation for ligand recognition [19–24]. While intracellular expression of TLR3 is mediated by its cytoplasmic linker region [22], the localization of TLR7 and TLR9 is controlled by their transmembrane domains [25,26]. Since TLR7 and TLR8 are phylogenetically very close and share similar transmembrane regions, it is likely that targeting of TLR8 to the intracellular compartment is mediated accordingly. Recognition of viral nucleic acids in this specialized endosomal compartment requires the uptake of virus particles or material from virus-infected cells. The release of TLR7 and TLR8 agonists from the endosomal cargo upon acidification is likely to be mediated by lysosomal enzymes [20,22]. The requirement for enzymatic digestion is an obvious explanation for intracellular recognition of nucleic acid ligands. In addition, restricting nucleic acid recognition to a specialized endosomal compartment may favour the recognition of viral over self-ligands as has been shown for TLR9 [26]. However, the intracellular localization of nucleic acid-sensing TLRs necessitates the presence of uptake mechanisms for virus and virus-infected cellular material mediated by moieties different from the TLR ligands themselves. Consequently, when using viral nucleic acid mimics such as imidazoquinolines and oligonucleotides alone or in combination with transfection reagents, the uptake of these synthetic TLR ligands is subject to other mechanisms and constraints and does not entirely reflect physiological processes.

The uptake of cellular material is regulated by a variety of so called “eat me” signals such as phosphatidylserine and other stress-induced molecules on the cell surface [27], which are