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Mini-review

Targeting pattern-recognition receptors to discover new small molecule immune modulators

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ABSTRACT

Pattern recognition receptors (PRRs) are key immune receptors of the innate immune system, which recognize the conserved pathogen-associated molecular patterns (PAMPs) of the invading pathogens. Compared to the adaptive immune receptors, PRRs have three distinguishing features, viz., universal expression, fast response and recognizing many kinds of microbes. Toll-like receptors (TLRs), RIG-I-like receptors (RLRs), C-type lectin receptors (CLRs) and NOD-like receptors (NLRs) recognize viral nucleic acid/bacterial fragments and trigger anti-microbial innate immune responses. Upon recognition of their ligand species, PRRs recruit specific intracellular adaptor proteins to initiate signaling pathways culminating in the activation of nuclear factor-κB (NF-κB), mitogen-activated protein (MAP) kinases and interferon regulatory factors (IRFs) that control the transcription of genes encoding pro-inflammatory factors including type I interferon and other inflammatory cytokines, which are critical for eliminating the potential threat to the host. Here, we summarize the effects of small molecule regulators acting on signaling pathways initiated by TLR, RLR and NLR as well as their influence on innate and adaptive immune responses leading to therapy.

1. Introduction

The innate immune system is the first-line barrier of the host defense against the invading microbes [1]. The innate immune cells (macrophages and dendritic cells) have sensors to recognize the specific pathogen-associated molecule patterns (PAMPs) to detect the pathogenic microorganisms. These sensors, so-called PRRs (especially TLR, CLR, RLR and NLR) distinguish the unique patterns of microbes by multi-receptor recognition and the dangerous signals transmitted by the host [2].

Upon recognition of PAMPs by TLRs, a group of intracellular signaling molecules is activated leading to the expression of NF-κB dependent pro-inflammatory cytokines and/or IRF-dependent type I interferon (IFNs). RLR is a member of the RNA helicase family of the DExD/H domain, which recognizes and regulates native viral replication and transmission by controlling the expression of type I IFN. NLR is composed of a large number of intracellular PRRs containing nucleotide binding oligomeric domain protein (NOD) and leucine-rich repeats (LRR). NOD-like receptor family pyrin domain (NLRP) is vital for the host defense against viral infections [3]. C-type lectin receptors, on the other hand, recognize complex

Abbreviations:: BIR, baculovirus inhibitor repeat; CARD, caspase recruitment domains; CFS, chronic fatigue syndrome; CLR, C-type lectin recceptors; CTD, connective tissue disease; CTL, cytotoxic T lymphocytes; DAMPs, damage associated molecular patterns; DC, dendritic cell; dsRNA, double-stranded RNA; ECDs, ecto-domains; HBV, hepatitis B virus; HCC, Hepatocellular carcinoma; iE-DAP, γ-D-glutamyl-meso-diaminopimelic acid; IFNs, type I interferons; IL-1, interleukin-1; LCMV, lymphocytic choriomeningitis virus; LGP2, laboratory of genetics and physiology gene 2; LRR, leucine rich repeat; LPS, lipopolysaccharide; MAL, MyD88-adaptor-like; MAP, mitogen-activated protein; MDA5, melanoma differentiation-associated protein 5; MDP, muramyl dipeptide; MHC, major histocompatibility complex; MyD88, myeloid differentiation factor 88; NF-κB, nuclear factor-κB; NOD, nucleotide-binding oligomerization domain; NLRs, NOD-like receptors; NTHi, nontypeable Haemophilus Influenzae; ODNs, Oligodeoxynucleotides; PAMPs, pathogen-associated molecule patterns; PRRs, pattern-recognition receptors; PYD, pyrine domain; PYRD, pyrindomain; RA, Rheumatoid arthritis; RLRs, RIG-I-like receptors; SARM, sterile alpha and HEAT/Armadillo motif protein; TBK1, TANK-binding kinase 1; TIRAP, TIR-associated protein; TLRs, Toll-like receptors; TRAM, Toll-receptor-associated molecule; TRIF, Toll-receptor-associated activator of interferon.

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carbohydrate structures present in the bacterial cell walls, fungal and viral surfaces [4]. Although the inherent signal can play a key role in host defense, the body still needs a more flexible regulatory network to ensure the accuracy of the PRR signals to effectively eliminate the invading pathogens while avoiding harmful immune pathology.

In this regard, many modulators such as phosphatases, ubiquitin-related proteins and transcription factors are involved in the regulation of targeted PRRs signaling. Recent studies have shown that host cells respond to intracellular dsDNA released by DNA viruses, bacteria or host cells to trigger type I IFN production [5]. In addition, the signals downstream of the PRRs are cross modulated either in a cooperative or an antagonistic manner. Since the fine specificity of ligands binding to CLRs are yet to be clarified, in this review, we focus on the recognition mechanisms of TLR, RLR and NLR and the role of small molecule discovery in recent years, as well as their influence on anti-inflammatory, anti-virus and anti-cancer responses. In these small molecule regulators, we will discuss the small molecules that have made rapid development or have substantial breakthroughs in recent years in detail, and those small molecules that have been extensively studied or reported elsewhere will only be mentioned briefly.

2. The discovery of PRRs (TLR, RLR, NLR)

In 1984, TLR was discovered to be a receptor protein from insects *Drosophila*. As early as 1989, Charles Janeway first proposed the concept of PRRs [6]. The basic structure of the TLR became gradually clear. TLR structure contains three conservative domains that participate in signal transduction. The structure of TLR2-TLR1, TLR2-TLR6, TLR3, TLR5, TLR7, TLR8 and TLR9 and other TLR-ligand complexes were found in the subsequent years. Today, the TLR family is consisted of more than 10 members of the human family. Most of them are located on the cell surface, while TLR3, 7, 8 and 9 are located in the cell membrane. TLR is involved in the identification of multiple ligands, including PAMP and damage associated molecular patterns (DAMPs). Cell surface TLRs can mediate binding to PAMPs by homo-/hetrodimerizing, for example TLR1/2 or TLR2/6 recognize lipoprotein, TLR3 recognize dsRNA, TLR4 and TLR5 recognize lipopolysaccharide (LPS) and flagellin, ssRNA activates TLR7 or 8 and CpG-ODN triggers TLR9, respectively [7].

The second set of PRRs is RLRs, which are located in the cytoplasm and recognize exogenous RNA and viral nucleic acids. RLRs contain retinoic acid-inducible gene-I (RIG-1), melanoma differentiation-associated protein 5 (MDA5) and laboratory of genetics and physiology gene 2 (LGP2) [8]. These three parts have the function of a ATPase. RIG-I and MDA5 contain the RNA helicase domain and two CARD-like domains that can trigger downstream signaling pathways, with the exception that LGP2 lacks the CARD-like domain. RIG-1 and MDA5 are able to recognize viral RNA with a high ligand specificity, causing transcription of type I IFN, which in turn induces nuclear localization of the transcription factors, IRF-3, IRF-7 and NF-κB. The C-terminal domain of RIG-I and LGP2 contains an inhibitory domain (RD) that allows the host cell to remain in a dormant state without the stimulation of viral RNA cells [9].

The third group of PRRs is NLRs. In 2011, Shao's team discovered a congenital immune receptor molecule NLRC4, which existed in dendritic cells. It can recognize the flagellin molecule present in the pathogen [10] and involved in the stimulation of immune functions leading to an intense inflammatory responses. Twenty-three NLR members have been identified in tissue cells so far, including monocytes, macrophages, T cells and B cells. They are divided into 3 subfamilies according to their N-domain viz., caspase activating and recruitment domain (CARD), pyrine domain (PYD) (NACHT-, LRR-, and NALPs) and baculovirus inhibitor repeat (BIR) [11].

3. TLRs and inflammation

3.1. Signaling pathway of TLRs

To a large extent, the characteristics of TLRs have been decrypted. It has four adapter proteins in the TIR (Toll/interleukin-1 receptor (IL-1R) homologous region) homologous domains: MyD88 (myeloid differentiation factor 88), MAL/TIRAP (MyD88-adaptor-like/TIR-associated protein), TRIF (Toll-receptor-associated activator of interferon) and TRAM (Toll-receptor-associated molecule). These related adapter proteins activate kinases that can interact with the transcription factors that are responsible for inflammatory responses. Though the function of SARM is not yet clear, it is nonetheless a highly conserved protein in terms of its structure. Studies have confirmed that SARM is a negative regulatory molecule that relies on TRIF signaling pathways. It has been shown that MyD88 overexpression significantly reduces NF-κB activation induced by non-typable *Haemophilus influenza* (NTHi), and also reduces the secretion of IL-6 and IL-1β induced by NTHi. Previous studies have shown that TRIF plays an important role in TLR4 agonist-assisted early T cell responses. It was also noted that TRIF signaling of TLR4 can control T cell activation. According to previous study, TRIF can independently activate CD86/CD40 on the DCs of spleen, but for the up-regulation of CD80, it requires engagement with not only TRIF but also MyD88 [12]. All the TLRs, TLR1, 2, 5, 6, 7, 8, 9 and 11 function by using MyD88 as the adaptor protein to effectuate signaling cascade, while TLR3 uses TRIF as the connector. Interestingly, TLR4 is unique in the sense that it can promote the downstream signaling immune reactions through both the MyD88 and TRIF adaptor molecules (Fig. 1). Recent studies have shown that TRIF is closely related to the activation of IRF3 and NF-κB signaling pathways. Hence, it is plausible that in TRIF mediated signaling pathway, activation of IRF3 can facilitate the synthesis of TNFα by linking with TNF promoter. But immune activation is a double-edged sword, and too powerful immune responses may also bring undesirable effects on the host including endotoxic shock and autoimmune diseases. Under steady state conditions, by the regulation of TLR signaling cascade should control this excessive immune reactions [13]. TLRs have a pivotal role in chronic inflammation like rheumatic diseases, including rheumatoid arthritis, systemic lupus erythematosus, gout and Lyme disease [14]. Moreover, an association of TLR polymorphisms with disease susceptibility has been well documented.

3.2. TLRs family members and their small molecule immune modulators

TLR1, 2 and 6: Previous publications have demonstrated that TLR2 activation can be used for the treatment of lung cancer and for the inhibition of breast and pancreatic cancer growth [15]. TLR1/2 agonists also have beneficial effects on chronic and acute inflammatory conditions and infectious diseases such as influenza, asthma and age-induced obesity [16]. The discovery of TLR2 selective modulators is however very challenging, mainly due to heterodimerization with either TLR1 or TLR6 before signaling initiated through TLR2 [17]. Pam2CSK4 and Pam3CSK4 (Table 1) are all TLR2 agonists, which could be used as potential candidates for vaccine adjuvants. The reference literature shows that the acyl groups with the optimal length (C16) and the appropriately oriented ester carbonyl group are necessary for the agonistic activity of TLR2, and often accompanied by a great deal of work and challenge in the development of small molecule modulators [18].

We have interest in researching small molecule agents targeting the protein-protein interactions [19], including the small molecule agonist (CU-T12-9) obtained from structure optimization guided by