Review Article

Immunomodulatory Effects of dsRNA and Its Potential as Vaccine Adjuvant

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dsRNA can be detected by pattern recognition receptors, for example, TLR3, MDA-5, NLRP3 to induce proinflammatory cytokines responsible for innate/adaptive immunity. Recognized by endosomal TLR3 in myeloid DCs (mDCs), dsRNA can activate mDCs into mature antigen presenting cells (mAPCs) which in turn present antigen epitopes with MHC-I molecules to naïve T cells. Coadministration of protein and synthetic dsRNA analogues can elicit an antigen-specific Th1-polarized immune response which stimulates the CD8⁺ CTL response and possibly dampen Th17 response. Synthetic dsRNA analogues have been tested as vaccine adjuvant against viral infections in animal models. However, a dsRNA receptor, TLR3 can be expressed in tumor cells while other members of TLR family, for example, TLR4 and TLR2 have been shown to promote tumor progression, metastasis, and chemoresistance. Thus, the promising potential of dsRNA analogues as a tumor therapeutic vaccine adjuvant should be evaluated cautiously.

1. Introduction

Pathogens on invading host cells express molecules that are broadly shared by all microbes and distinct from host. These include lipopolysaccharide, peptidoglycan, flagellin and microbial nucleic acids, and collectively are referred to as pathogen-associated molecular patterns (PAMPs) [1–3]. Pattern recognition receptors (PRRs) of the host when recognizing PAMPs trigger a release of inflammatory cytokines and type I interferons (IFNs) [4, 5]. PRRs are evolutionally conserved and have been investigated extensively [4]. From the initial investigation of Toll-like receptor (TLR) family [6] to the recent discoveries of retinoic acid-inducible gene-I (RIG-I)-like receptors (RLRs) [7, 8] and the nucleotide-binding domain and leucine-rich repeat containing gene family (NLRs, also known as NOD-like receptors) [9, 10], all the evidence point to an important role in host defense. TLRs are membrane bound receptors that sense PAMPs on the cell surface or in endosomes [11] while RLRs and NLRs recognize microbial molecules in the host cytosol

[12]. The individual members of these PRRs families are characterized by their ligand specificity, cellular localization and activation of unique downstream signaling pathways. Immunity against different microbial pathogen primarily depends on the recognition of the specific PAMP of the pathogen by the corresponding PRR.

Double-stranded RNA (dsRNA), a virus replication intermediate and a signature of infection, is sensed by TLR3, two members of RLRs family, that is, the RNA helicases RIG-I and melanoma differentiation-associated gene 5 (MDA-5), and the NLR pyrin domain (NLRP) 3 protein of NLR family. These trigger the release of inflammatory cytokines, that is, activating innate immunity which shapes adaptive immune response [11, 13, 14]. The primary cytokines involved in this response are type I IFNs including IFN- α and IFN- β [15].

In this review, the mechanism of innate and adaptive immunity induced by dsRNA and the potential application of dsRNA as a vaccine adjuvant against viral infection and anticancer immunotherapy is elaborated.

2. dsRNA Induced Signaling

2.1. The TLR3-Mediated Signaling. TLRs are type I integral membrane glycoproteins which have a trimodular structure composed of an extracellular domain, a single transmembrane domain and an intracellular Toll/IL-1 receptor (TIR) domain [11, 16, 17]. Twelve members of TLR family have been identified in mammals [11]. The extracellular N-terminal domains of TLRs contain 16–28 leucine-rich repeats (LRRs) [16, 18] in a horse-shoe structure [17]. Each individual LRR is composed of 20–30 amino acids with a conserved characteristic repetitive sequence pattern rich in leucines, the “LxxLxLxxNxL” motif and two or more repeats in tandem, form curved solenoid structures suitable for protein-protein interactions [19]. The extracellular domain of human TLR3 comprises of 23 LRRs in a horse-shoe shaped structure. The convex face of the extracellular domain of TLR3 is glycosylation-free and contains many positively charged residues while the concave face is largely glycosylated and negatively charged [20, 21]. The dsRNA binding site of TLR3 is located in two regions near the N-terminus and C-terminus. When dsRNA interacts, two ectodomains of TLR3 are connected by dsRNA in an “m” shape to form a TLR3-dsRNA 2:1 complex [22]. When combined with TLR3, dsRNA spans the whole “m” consisting of two “horse-shoes” of the ectodomains of TLR3 (Figure 1A). This satisfies the minimum requirement of 40–50 base pairs of dsRNA allowing stable binding to TLR3 inducing signaling [16, 23, 24]. With the secondary structure, the transmembrane domain of TLR3 is a single α -helix and the endodomain is composed of a five-stranded β -sheet surrounded by five α -helices that forms the TIR domain [25]. The B-B loop that connects β -strand B with α -helix B in the TIR domain is considered the essential structure that directly interacts with the adaptor protein TIR domain-containing adaptor inducing IFN- β (TRIF) [26]. In addition to the B-B loop, three boxes of conserved residues that reside in TIR domain are involved in TLR3 signaling [25, 27].

TLR3 can be found both intracellularly and on the cell surface in human fibroblasts and epithelial cells. However, it is predominantly located in intracellular vesicles, for example, endosomes, in most cell types including dendritic cells and macrophages [28, 29]. TLR3 is activated by extracellular dsRNA that is released from dsRNA viruses or is produced during single-stranded RNA viruses' replication or comes from application of synthetic dsRNA analogues [4]. It is largely unknown how extracellular dsRNA are delivered to the intracellular vesicles containing TLR3. Studies have suggested that CD14 may play an important role in dsRNA uptake [20, 30]. Once internalized into the endosome, dsRNA binds to its adaptor protein TLR3 and activates several signaling pathways. Upon binding to dsRNA, the B-B loop of the TLR3 TIR domain combines with TIR domain of TRIF activating several transcription factors, including nuclear factor- κ B (NF- κ B), interferon regulatory factor 3 (IRF3), and activating protein 1 (AP-1) [25].

There are two pathways to activate NF- κ B mediated by receptor-interacting protein 1 (RIP1) and tumor necrosis

factor (TNF) receptor-associated factor 6 (TRAF6) (Figure 1A). TRAF6 is a ubiquitin ligase and plays a role in RIP1 polyubiquitination [31, 32]. Polyubiquitinated RIP1 is recognized by ubiquitin receptors, the transforming growth factor β -activating kinase (TAK) binding protein (TAB) 2 and 3, which in turn activate TAK1 [33]. I κ B kinase-related kinase α (IKK α) and IKK β are phosphorylated by the activated TAK1. This leads to phosphorylation and degradation of I κ B, an inhibitor of NF- κ B, and eventually results in the translocation of NF- κ B to cell nucleus. This is followed by the activation of specific gene promoter A20. TAK1 also activates the 2 other classes of kinase, JNK and p38 and these activate the family of AP-1 transcription factors [27]. The third signal comes from TRIF activating IRF3 (Figure 1A). TRIF activates the kinase complex TRAF family member-associated NF- κ B activator (TANK)-binding kinase 1 (TBK1) and IKK ϵ through its adaptor protein, NF- κ B activating kinase (NAK)-associated protein 1 (NAP1), leading to the phosphorylation and nuclear translocation of IRF3 consequently inducing the expression of IFN- β [34]. In addition to NAP1, TRAF3 is also part of the TBK1/IKK ϵ complex that is involved in the TRIF-mediated IRF3 activation [35]. Another signal from TLR3 is related to the phosphorylation of two specific tyrosine residues (Tyr⁷⁵⁹ and Tyr⁸⁵⁸) in the TLR3 TIR domain when TLR3 interacts with TRIF (Figure 1A). Phosphorylated Tyr⁷⁵⁹ recruits phosphatidylinositol 3-kinase (PI3K) which then activates kinase Akt required for phosphorylation and activation of IRF3 in nucleus [36]. The phosphorylation of Tyr⁷⁵⁹ and also Tyr⁸⁵⁸ results in degradation of I κ B leading to NF- κ B release and this induces phosphorylation of NF- κ B which partially activates it [37]. Tyrosine kinase c-Src also plays a role in Akt activation [38]. The unique signal pathway of TRIF is able to induce mammalian cell apoptosis (Figure 1A). TRIF interacts with Fas-associated cell death domain (FADD) protein through RIP1 which in turn activates procaspase-8 to initiate cell apoptosis [25, 39].

2.2. The RLRs-Mediated Signaling. RLRs are cytoplasmic viral RNA sensors with three recognized members including RIG-I, MDA-5 and laboratory of genetics and physiology 2 (LGP2). All belong to the DEXD/H box RNA helicase family [40]. In addition to the central helicase domain, RIG-I and MDA-5 have two caspase recruitment domains (CARDs) at its N-terminus which are responsible for downstream signaling cascade through the interaction with a CARD-containing adapter, mitochondrial antiviral signaling adapter (MAVS, also known as IPS-1, VISA, or Cardif) located in the outer mitochondrial membrane [41]. LGP2, lacking the N-terminal CARD, works as a negative regulator of RLRs signaling. Although only a single CARD physically interacts with MAVS, both CARDs are essential for downstream signaling [42, 43]. The RIG-I activation is self-inhibited by the C-terminal regulatory domain (RD), also referred as the C-terminal domain (CTD) or repressor domain, through intramolecular association between RD and both the CARD and the helicase domains [42–44]. RD is also responsible for the binding affinity to the dsRNA and 5'-triphosphated single-stranded RNA (5'ppp-ssRNA) of viral RNAs with

FIGURE 1: dsRNA induced signal pathways. (A) *dsRNA signaling through TLR3*. dsRNA that is internalized into endosome binds to TLR3. The mechanism of dsRNA internalization is unknown, CD14 may play a role. TLR3 possesses two dsRNA binding sites near the N-terminus and C-terminus. When combined with dsRNA, four dsRNA binding sites from two TLR3 molecules that are linked by the sole dsRNA molecule in an “m” shape, and the B-B loop of the TLR3 TIR domain combines with the TIR domain of TRIF. The interaction of TRIF with RIP1 or TRAF6 results in polyubiquitination of RIP1, the latter binds ubiquitin receptors TAB2 and TAB3 which activates TAK1. Activated TAK1 induces phosphorylation of IKKα and IKKβ leading to phosphorylation and degradation of IκB. The release and translocation of NF-κB to cell nucleus then occurs which results in the specific gene promoter A20 being activated. TAK1 also starts an activation sequence beginning with JNK and p38, which activates c-JUN and ATF2 and this activates the AP-1 transcription factors family. TRIF activates TBK1 and IKKε through NAPI and this results in phosphorylation and nuclear translocation of IRF3 resulting in IFN-β production. TRAF3 also binds with the TBK1/IKKε complex inducing IRF3 activation. Combination of TRIF results in phosphorylation of Tyr⁷⁵⁹ and Tyr⁸⁵⁸ in the TLR3 TIR domain which subsequently induces the phosphorylation of, and degradation of IκB leading to NF-κB release. Phosphorylated Tyr⁷⁵⁹ recruits PI3K and activates kinase Akt for full phosphorylation and activation of IRF3 in nucleus. Tyrosine kinase c-Src also plays a role in Akt activation. TRIF interacts with FADD through RIP1 and activates procaspase-8 to initiate cell apoptosis. (B) *dsRNA signaling through MDA-5*. dsRNA is recognized by MDA-5 in cytosol. MDA-5 interacts with MAVS located on the outer mitochondrial membrane via CARD-CARD interaction leading to the dimerization of the MAVS N-terminal CARD domains which subsequently binds TRAF3 directly through the interaction between the TRAF domain of TRAF3 and the TRAF-interacting motif in the PRR of MAVS. Then the RING domain of TRAF3 forms a scaffold to assemble the noncanonical IKKs signal complex composed of TANK, TBK1, IKKε, NAPI and NEMO. This complex activates the signal-dependent phosphorylation of IRF3 and IRF7 to form a functional homodimer or heterodimer which translocate to the nucleus to induce expression of type I IFN. TRAF2 and TRAF6 also associates with MAVS and is involved in activation of P38 MAPK and promotion of IL-12 and type I IFN production or activation of NF-κB, JNK and P38 signaling. In addition, MAVS recruits TRADD and interacts with TRAF3, TANK, FADD and RIP1 to activate both IRF3 and NF-κB signaling. (C) *dsRNA signaling through NLRP3 inflammasome*. NLRP3 recognizes dsRNA by a series of LRRs at the C-terminus. The NACHT domain at the middle of NLRP3 is related to self-oligomerization and the formation of inflammasome. Studies have suggested dsRNA may activate the NLRP3 inflammasome. Activation of NLRP3 results in the interaction of NLRP3 PYD with ASC PYD, and in turn ASC CARD associates with pro-caspase-1 CARD and thereby assembles the NLRP3 inflammasome. Once incorporated into NLRP3 inflammasome, pro-caspase-1 is activated by proteolytic cleavages to remove the CARD prodomain. The active caspase-1 in turn cleaves the IL-1β, IL-18, IL-33 and IL-1F7 precursors into their active forms and these active cytokines are secreted extracellularly.

a common core RNA binding site specifically adapted to distinct and unique patterns [44–47]. It is also responsible for RIG-I dimerization. MDA-5 RD preferentially binds dsRNA with blunt ends but does not associate with dsRNA with either the 5′ or 3′ overhangs [13]. The central helicase domain displays cooperative RNA binding properties [43, 48, 49]. The RD of MDA-5 does not exhibit self-inhibitory activity [42, 50]. MDA-5 is negatively regulated by dihydroxyacetone kinase (DAK) and other possible regulators [51].

RIG-I binds 5′-triphosphate RNA in single- or double-stranded forms [52–54] or short dsRNA of 300–1000 bp

without a 5′-triphosphate [54] while MDA-5 recognizes long dsRNA of more than 1000 bp in length and the synthetic dsRNA analogue polyinosinic-polycytidylic acid [poly (I:C)] [54–56]. Upon sensing RNA, both RIG-I and MDA-5 are activated and initiate downstream signaling through the common pathway via adaptor protein MAVS (Figure 1B). MAVS consists of a CARD at the N-terminus, a proline-rich region (PRR) in the middle and a transmembrane domain at the C-terminus attached to the outer surface of mitochondria [57]. Activated RIG-I and MDA-5 associate MAVS via a CARD-CARD interaction which leads to the

dimerization of the MAVS N-terminal CARD domains. Once activated, binding to TRAF3 occurs directly through the interaction between the TRAF domain of TRAF3 and the TRAF-interacting motif (TIM) in the PRR of MAVS [58–60]. Following the association of TRAF3 with MAVS, the RING domain of TRAF3 forms a scaffold to assemble noncanonical IKKs signal complex composed of TANK, TBK1, IKK ϵ , NAP1 and NF- κ B essential modulator (NEMO). This complex then activates the signal-dependent phosphorylation of IRF3 and IRF7 to form a functional homodimer or heterodimer and translocates to the nucleus [61, 62]. The association of MAVS and TRAF2 or TRAF6 has also similarly been demonstrated [63]. TRAF6 is essential in the activation of NF- κ B, JNK and P38 signaling [64] while TRAF2 is involved in activation of P38 MAPK which promotes IL-12 and type I IFN production [65]. Moreover, TNFR-associated death domain (TRADD) is recruited to MAVS following virus infection and interacts with TRAF3, TANK, FADD and RIP1 as well as activating both IRF3 and NF- κ B signaling [66]. Another protein-termed stimulator of interferon genes (STING, also known as MITA or MYPS) expressed either on the outer mitochondrial membrane [67] or endoplasmic reticulum [68] directly interacts with RIG-I but not MDA-5. It subsequently recruits TBK1 activating IRF-3. STING has been reported to associate with major histocompatibility complex class II (MHC II) and mediates apoptotic signals via ERK activation [69]. It was recently reported that RIG-I binds to an adaptor apoptosis-associated speck-like protein containing a caspase-activating and recruitment domain (ASC) to trigger caspase-1-dependent inflammasome activation by a mechanism independent of MAVS, CARD9 and NLRP3. This suggests that RIG-I is able to activate the inflammasome in response to certain RNA viruses [70].

2.3. The NLRs-Mediated Signaling via Inflammasome. NLRs are intracellular PRRs sensing PAMPs and danger signals or danger-associated molecular patterns (DAMPs) released by injured cells [71]. The NLR family has 23 members in humans and at least 34 members in mice [72]. NLRs are multidomain proteins with tripartite structure composed of an N-terminal effector region, a central NACHT (Neuronal Apoptosis inhibitory protein, NAIP; Class II transactivator, CIITA; plant Het product involved in vegetative incompatibility, HET-E; Telomerase-associated protein 1, TP-1) domain (also known as NOD domain), and a C-terminal region for PAMPs recognition. The C-terminal region is characterized by a series of LRRs and is implicated in ligand sensing and NLRs autoregulation but the precise mechanism is not clear [71]. The NACHT domain is a member of signal transduction ATPases, part of the P-loop NTPase family [73] and is related to self oligomerization and the formation of inflammasome [71]. The N-terminal region contains several protein interaction modules, such as acidic domain, baculoviral inhibitory repeat (BIR)-like domain, CARD and pyrin domain (PYD). Accordingly, NLRs are further divided into subfamilies as NLRA, NLRB, NLRC and NLRP. An additional subfamily, NLRX, is characterized by the presence of an N-terminal domain with no strong

homology to any known domains of other NLR subfamily member [74].

Upon recognition of PAMPs, toxins, or danger signals by NLRs, a large protein complex termed the inflammasome composed of NLRs, ASC and pro-caspase-1 is activated. This protein platform activates pro-caspase-1 into its active form of caspase-1 and this hydrolyzes pro-IL-1 β and pro-IL-18 into their mature biologically active forms which are secreted extracellularly to play a role in immune response [14, 71]. Limited studies have suggested dsRNA is an activator of NLRP3 inflammasome [75–78] although this has been disputed [79]. NLRP3 senses PAMPs either from bacteria, such as lipopolysaccharide (LPS), muramyl dipeptide (MDP), bacterial pore-forming toxin and bacterial DNA and RNA, or from viruses such as viral ssRNA or dsRNA, dsRNA analogue poly (I:C). It also senses other compounds such as imidazoquinoline antiviral drugs R837 and R848, nonmicrobial signals encompassing uric acid crystals, calcium pyrophosphate dehydrate (CPPD), asbestos, silica, extracellular ATP, alum adjuvant and fibrillar amyloid- β [14, 71, 72, 80]. It is believed that NLRP3 activation results in the interaction of NLRP3 PYD with ASC PYD which in turn causes ASC CARD to associate with pro-caspase-1 CARD assembling the NLRP3 inflammasome (Figure 1C) [81]. Besides NLRP3, ASC and pro-caspase-1, human NLRP3 inflammasome contains a CARD-containing protein, CARD inhibitor of NF- κ B-activating ligands (Cardinal, also known as CARD8). However, there is no homologue of Cardinal in the mouse [81–83]. Allen et al. (2009) utilized small heteroduplex RNA (shRNA) to knockdown the expressions of ASC, NLRP3 and Cardinal respectively in human THP-1 monocyte cell lines. The IL-1 β production triggered by lentivirus infection was significantly attenuated by the addition of shRNA of ASC or NLRP3. In contrast, secretion of IL-1 β was not affected by knockdowns of Cardinal. Thus, it has been suggested that Cardinal may not affect NLRP3 inflammasome function [77]. Furthermore, Cardinal does not bind to NLRP3 in the inflammasome [84].

The activation of the NLRP3 inflammasome has been demonstrated through a number of mechanisms. However, the direct ligand for NLRP3 has yet to be defined. It has been proposed that the activation of the NLRP3 inflammasome requires both microbial molecules and a second signal such as extracellular ATP or pore-forming molecules [72]. Alternatively, it has also been suggested that reactive oxygen species (ROS) may be the common NLRP3 inflammasome activator since the most striking features associated with NLRP3 activators like potassium efflux and the induction of frustrated phagocytosis all leads to ROS production via NADPH [71, 85, 86]. In particular, the activation of NLRP3 inflammasome triggered by virus infection or by poly (I:C) requires sensing viral RNA or poly (I:C), lysosomal maturation, cathepsin B and ROS generation [77]. The assembly of NLRP3 inflammasome leads to the activation of pro-caspase-1 and consequently the maturation of pro-IL-1 β and pro-IL-18 (Figure 1C).

Caspases belong to a conserved metazoan aspartate-specific cysteine proteases family with 11 members in human (caspases 1 to 10, and 14) and 10 members in murine species

(caspases 1, 2, 3, 6, 7, 8, 9, 11, 12, and 14) [87, 88]. All caspases are synthesized as inactive zymogens containing a prodomain and are divided into two subfamilies: initiator caspases and effector caspases based on the length of their prodomains. Initiator caspases (caspases 1, 2, 4, 5, 8, 9, 10, 11, 12) are involved in the interaction with upstream adapter molecules and possess long prodomains that contain either the death effector domain or CARD. Effector caspases (caspases 3, 6, 7) that possess short prodomains are activated by upstream caspases and are able to cleave multiple cellular substrates involved in apoptosis [87]. Caspase-1, along with caspases 4, 5, 11, and 12 are often referred to as proinflammatory caspases. Once incorporated into NLRP3 inflammasome, pro-caspase-1 is activated by proteolytic cleavage to remove the CARD prodomain. The active caspase-1 in turn cleaves the IL-1 β , IL-18, IL-33 and IL-1F7 precursors into their active forms and these active cytokines are secreted extracellularly and become immunoreactive (Figure 1C) [89].

It was recently reported that influenza virus infection results in the activation of NLR inflammasomes in the lung. Although NLRP3 is required for inflammasome activation in certain cell types, adaptive immunity to influenza virus is ASC and caspase-1 dependent rather than NLRP3 dependent, suggesting a central role of ASC inflammasomes. The investigators concluded that influenza virus infection stimulates NLRP3-dependent and NLRP3 independent inflammasomes in a cell type-specific manner [90]. Moreover, it has been suggested that some viral RNA can activate inflammasome via interaction between RIG-I and adaptor ASC independent of NLRP3, MAVS, and CARD [70].

3. Modulation of Adaptive Immunity by dsRNA

3.1. Activation of DCs. Activation of dendritic cells (DCs) occurs upon sensing dsRNA [91]. DCs express a repertoire of PRRs including TLRs (TLR3 is not expressed in plasmacytoid DCs), RIG-I and MDA-5 (absent in plasmacytoid DCs), as well as NLRs, and are able to recognize a range of pathogenic microbes [92–94]. The interaction of PAMPs and PRRs on DCs induces the maturation and activation of DCs via transcription, translation and secretion of inflammatory cytokines and chemokines through the signal pathways as described above. The activated DCs, characterized by enhanced antigen presentation capacity and referred to as antigen-presenting cells (APCs), migrate to draining lymph nodes and interact with T and/or B lymphocytes initiating the immunity process. Among the cytokines triggered and secreted, type I IFN plays a major role in the cross-priming of CD8⁺ T-cells by promoting the expression of costimulatory molecules of DCs [95]. The proliferation and differentiation of the lymphocytes are mediated by signals from the activated DCs which comprise of the co-presentation of MHC molecules and pathogen-derived peptides. Additionally, signals from costimulatory molecules including CD80 and CD86, as well as the instructional signals, for example, IL-12p70 for Th1, IL-4 for Th2, and

IL-6 and IL-23 for Th17 from the presenting DCs [91] are also present. dsRNA receptors which include TLR3 and RIG-I/MDA-5 are expressed in myeloid DCs (mDCs) and primarily produce IL-12 and IFN- β when recognition of dsRNA occurs [94]. However, poly (I:C) with different molecular weights have differential effects on the maturation of DCs [96]. Interestingly, co-culture of bone marrow-derived DCs with protein and poly (I:C) reduced the antigen uptake by DCs. However, the reduced uptake of antigen did not affect CTL priming by DCs suggesting that the reduction in uptake of soluble antigen in the presence of poly (I:C) is independent of TLR-mediated DC activation [97].

3.2. Activation of Th1 Cells. Newly primed CD4⁺ T-cells are programmed by various cytokines and other factors from DCs and other innate immune cells to differentiate into Th1 or Th2 or Th17 effector cells or regulatory T-cells (Treg) [98]. Th1 lymphocytes are produced by the naïve CD4⁺ T-cells (Th0) interacting with IL-12 from mDCs to stimulate the expression of signal transducer and activator of transcription (STAT) 1 and subsequently that of T box expressed in T-cells (T-bet). The latter is the critical transcription factor of Th1 cells [99]. Activated Th1 cells produce cytokines like IL-2 and IFN- γ that are cofactors in CD8⁺ CTLs activation and synergistically activate mDCs acting via a feedback loop. Signals from Th1 cells are essential for CD8⁺ T-cell proliferation and function [99]. It is believed that dsRNA is capable of inducing robust IL-12p70 production which reduces the threshold of Th1 response and herein promotes Th1-biased adaptive immunity through TLR3 and JNK pathways [98, 100]. TNF- α , type I IFN and IL-18 also play important roles in the induction of Th1 response by dsRNA [101]. Type I IFN can activate mDCs directly by inducing phenotypic maturation which includes but is not restricted to upregulation of MHC Class I, class II, CD40, CD80, CD86 and higher expression of CD83 [102]. Besides inducing mDCs maturation and activation, IFN- α/β upregulates the expression of chemokine receptor CCR7 to sensitize mDCs to CCL19 and CCL21 which promote the migration of mDCs from peripheral tissues towards the T-cell area of lymphoid organs [102]. Type I IFN is also necessary for the generation of a Th1 CD4⁺ adaptive T-cell response whereas IL-12p40 and type II IFN are not. Therefore, the activation of Th1 cell response induced by dsRNA is possibly mediated by its capacity of inducing robust type I IFN production [92]. However, at low concentrations of dsRNA (0.1–1 μ g/ml), human lymphocytes express prototypic Th2 cytokine IL-4 [103]. Indeed, when coadministered with protein antigen, in addition to the induction of robust Th1 biased immunity, dsRNA is also capable of enhancing Th2 antigen-specific immune response [104–106].

3.3. Activation of Cytotoxic T Lymphocytes. When stimulated by dsRNA along with specific antigen, activated DCs are able to induce antigen-specific CD8⁺ cytotoxic T lymphocytes (CTLs) activation through cross-presentation and cross-priming mechanism [91]. During viral infection of mDCs, dsRNA is produced during replication in the infected mDCs

and the latter in turn activates mDCs through TRIF, MAVS, NLRP3 inflammasome and possibly another undefined signal pathway(s) to become APCs. APCs present endogenous antigens including those from intracellular viral origin with MHC class I molecules to naïve CD8⁺ lymphocytes along with costimulatory factors and instructional signals to activate the lymphocytes becoming MHC-I restricted CTLs. However, in most cases, the virus does not invade mDCs directly but instead infects cells other than mDCs. In such cases, the extrinsic viral antigen and dsRNA can be taken up into mDCs and these mDCs in turn present the antigen epitope with MHC-I molecules to CD8⁺ lymphocytes inducing a CTL response. This mechanism is referred to as cross-priming and inducible by TLR3-TRIF signaling and MDA-5-MAVS signaling [107, 108]. Type I IFN produced through these signal pathways also enhances the cross-priming ability of mDCs possibly via augmenting their capacity to deliver costimulatory signals [102] or directly stimulation of CD8⁺ T-cells [109]. The mechanism of mDCs uptake of the extrinsic antigens involves phagocytosis of particulate antigen, pinocytosis of soluble antigen and receptors mediating cross-presentation such as Fc receptor, mannose receptor and Dectin-1 [110]. The ability of cell-associated poly (I:C) with antigen to induce robust cross-priming responses in naïve mice is completely lost in the Tlr3-deficient chimera mice [107]. Immunized with protein along with alum and poly (I:C), the expansion of antigen-specific CD8⁺ T-cell can be reduced in both MAVS-deficient and TRIF-deficient mice and entirely abrogated in the bideficient mice [108]. Type I IFN produced through TRIF or MAVS signal pathway also enhances cross-priming by mDCs [102, 109]. A recent report suggested that CD8⁺ T-cells can be activated by dsRNA directly triggering TLR3 [111]. Priming of IFN- γ -producing CD8⁺ T-cells by dying tumor cells failed in the absence of a functional IL-1 receptor 1 and in Nlpr3-deficient or caspase-1-deficient mice unless exogenous IL-1 was present [112]. This suggests that the NLRP3 inflammasome is involved in the activation of CTLs.

3.4. Activation of Th17 Cells. Th17 cell is a proinflammatory lymphocyte belonging to Th cell subset [113, 114]. This subset preferentially produces IL-17, IL-17E, IL-22, and IL-21, but not IFN- γ or IL-4 [115]. Naïve T-cells are induced by transforming growth factor (TGF)- β to differentiate into two reciprocal subsets, that is, Th17 cells and Treg cells under different polarizing signals. IL-6 is the polarizing signal of Th17 which switches the transcriptional program initiated by TGF- β to induce the development of Th17 cells and blocks the development of Treg cells [116–118]. IL-23 plays a role in amplifying and stabilizing the phenotype of Th17 cells [119]. However, receptors for IL-23 are not expressed in naïve T-cells. In fact, Th17 cells can express such receptors if induced by TGF- β acting through ROR- γ t which is a unique transcription factor of Th17 (the human counterpart of murine ROR- γ t is ROR-c) [120]. ROR- γ t is the key transcription factor that orchestrates the differentiation of Th17 effector cell lineage by inducing transcription of the IL-17 gene in naïve helper T-cells and is also required for the

development of IL-17 producing cells in the presence of IL-6 and TGF- β [121]. IL-6 is involved in upregulation of IL-23R mRNA expression, and IL-6 and IL-23 synergistically augment its protein expression [122]. Therefore, IL-23 acts on T-cells that are already committed to the Th17 lineage rather than inducing Th17 differentiation.

Th17 cells are engaged in the neutrophil related inflammation against infections of fungi and certain extracellular bacteria [115]. Most parenchymal cells express IL-17 receptors that interact with IL-17 that are expressed primarily from Th17 cells to produce proinflammatory factors such as IL-1, IL-6, IL-8, tumor necrosis factor (TNF) and matrix metalloproteinases [115, 123]. Th17 cells can also induce chemokine production which attracts numerous effector T-cells into inflammatory area promoting the inflammatory response. Thus, inappropriate regulation of Th17 cells activities is associated with chronic inflammation and severe immunopathologic conditions such as autoimmunity [115]. Various studies have suggested that TRIF signal pathway is involved in Th17 response. TRIF signaling in mDCs might induce IL-12 and IL-23 production and play a role in Th17 activation [124]. However, the TLR3 pathway activated by dsRNA induces activation of IRF3 and IRF7 which exclusively induce p35 and p28 but not p19. These in turn would induce IL-12 (p35 and p40) and IL-27 (Epstein-Barr virus-induced gene 3 and p28) but not IL-23 (p19 and p40) [125]. Stimulation of endosomal TLR3 by poly (I:C) can induce mDCs to produce both IL-12p70 and IL-27; the former promotes Th1 cells to produce IFN- γ which can inhibit Th17 cells generation and the latter inhibits Th17 cells differentiation in a STAT1-dependent manner. Thus, poly (I:C) is likely to dampen Th17 responses [126]. TRIF-dependent type I IFN production along with its downstream signaling pathway negatively regulates Th17 development and constrains Th17-mediated autoimmune inflammation in mice [127]. It has also been reported that poly (I:C) can induce synthesis of both IL-17 and IL-21 and drive the differentiation of naïve Th cells into an IL-21 but not into an IL-17-producing phenotype without affecting the levels of transcription factors T-bet, GATA-3, or retinoic acid receptor-related orphan receptor C [128]. Thymic stromal lymphopoietin (TSLP) is a hemopoietic cytokine capable of conditioning mDCs and orientating the differentiation of naïve T-cells towards a Th2 profile. mDCs activated by a combination of TSLP and poly (I:C) are capable of priming naïve CD4⁺ T-cells to differentiate into Th17-cytokine-producing cells with a central memory T-cell phenotype without changing the Th2 polarization property of TSLP [129].

4. Potential Application of dsRNA as a Vaccine Adjuvant

4.1. The Adjuvant Properties of dsRNA. Innate immunity shapes adaptive immunity. Activated immune cells present specific antigen epitope associated with MHC-I/II molecules along with costimulatory and instructional signals to naïve T-cells to stimulate activation, differentiation

and proliferation of immunoreactive T-cells. As a potent activator of both innate and adaptive immunity, dsRNA simultaneously administered with a foreign antigen can act as an immunoadjuvant to induce specific adaptive immunity against the foreign antigen [91, 130].

Upon dsRNA stimulation, type I IFN production by DCs is critical for the adjuvant property of poly (I:C) [92]. Type I IFN is considered to be the major player linking innate to adaptive immunity. Besides activating DCs in an autocrine or paracrine manner, type I IFN is capable of inducing an antigen-specific CD8⁺ T-cell response, a CD4⁺ Th1 cell response and enhances the primary antibody response [2].

Salaun et al. (2009) demonstrated that proliferation and IFN- γ production of antigen-specific CD8⁺ T-cells in the mice immunized by antigen GP33 (an H-2D^b restricted peptide derived from Lymphocytic Choriomeningitis virus glycoprotein 33–41) with poly (I:C) adjuvant is abrogated when this regimen is administered in TLR3^{-/-} mice [131]. The mRNA of TLR3 is undetectable in either CD8⁺ effector or CD8⁺ effector memory T-cells. CD8⁺ T-cell proliferation and the ability of IFN- γ production are not affected by direct stimulation with poly (I:C) and specific TCR. Therefore, it appears that the adjuvant effect of poly (I:C) may be TLR3-dependent without any direct effect on CD8⁺ T-cells [131]. However, Ngoi et al. (2008) experimenting on (C57BL/6) mice that were TLR3^{-/-} and TRIF-deficient, with the poly (I:C) (InvivoGen) but using a different antigen, staphylococcal enterotoxin A, showed that CD8⁺ T-cells expansion was not impaired, that is, a type I IFN production in response to poly (I:C) occurred [132]. Splenocytes from naïve wild-type mice can produce IL-10 in a dose-dependent manner upon stimulation with poly (I:C) in the absence of antigen while IL-10 production was impaired in TLR3^{-/-} mice. Although these IL-10 producing cells may be innate immune cells, the IL-10 produced acts as a suppressive signal for adaptive immunity. Thus, the presence of TLR3 may suppress the development of adaptive immunity [132]. Indeed, the activation of NK cells, involved in innate immunity, is inducible by poly (I:C) via both MAVS- and TRIF-dependent pathways [133]. Kumar et al. [108] reported that in MAVS-deficient or TRIF-deficient mice immunized with OVA, alum and poly (I:C), the antigen-specific CD8⁺ T-cell expansion was reduced in either MAVS-deficient or TRIF-deficient mice and was entirely abrogated in the doubly deficient mice. Hence, the adjuvant effects of poly (I:C) requires a cooperative activation of TLR and cytoplasmic RNA helicase pathways [108]. Contamination of proteins used as antigens in these studies with other TLR ligands [93] or the contamination of CD8⁺ T-cells with other immune cells like innate cells might be a possible explanation of the discrepancy.

With respect to human CD8⁺ T-cells, TLR3 mRNA expression has been detected in human effector and effector memory cells but not in naïve and central memory T-cells [134]. The addition of poly (I:C) significantly increased the quantity of IFN- γ released by effector and/or effector memory CD8⁺ T-cells in response to PHA in a dose-dependent manner. However, poly (I:C) by itself did not detectably induce IFN- γ release by any of the purified CD8⁺

T-cell subsets. Furthermore, the addition of poly (I:C) had no effect on the cytolytic activity of CTL. Therefore, it is likely that the adjuvant effects and the corresponding mechanism of poly (I:C) are different in human and mouse cells.

4.2. dsRNA as Vaccine Adjuvant against Viral Infection. Lau et al. (2009) reported that mice vaccinated with H5N1 influenza vaccine with PIKA (a stabilized dsRNA) as adjuvant experienced a maximum three-fold increase in antibody titer comparable to that produced by mice immunized by vaccine with complete Freund's adjuvant [135]. Vaccination significantly reduced the virus titer in the lung of mice challenged after immunization with the H5 vaccine and PIKA adjuvant. Without a specific vaccine, sole PIKA administration was also capable of reducing pulmonary viral titer in mice [135]. This immunoprotective property of dsRNA against influenza virus was further demonstrated by other studies using another synthetic dsRNA analogue poly ICLC comprising of poly (I:C) stabilized with L-lysine and carboxymethylcellulose [136, 137]. PIKA was also able to induce activation and proliferation of B cells and NK cells. When HBsAg was coadministered, an increase in HBsAg-specific IgG production was noted. However, PIKA did not activate CD4⁺ and CD8⁺ T-cells [138]. Nonetheless, most studies suggested that poly (I:C) based dsRNA analogues displayed Th1 adjuvant property capable of activating antigen specific CD4⁺ and CD8⁺ T-cells when administrated as vaccine adjuvant [92, 105, 131, 132, 139, 140]. Another dsRNA analogue, poly (I:C12U) (Ampligen), was effective in inducing mDCs maturation and promoted Th1 cytokine IL-12 production while significantly decreased suppressive cytokine IL-10 production compared to that induced by poly (I:C) in healthy donors [140]. Intranasal administration of an Ampligen adjuvanted H5N1 vaccine resulted in the secretion of vaccine-specific IgA and IgG in nasal mucosa and serum respectively and protected mice against homologous and heterologous viral challenge [141]. Immunization of mice by hepatitis C virus nonstructural protein 3 and poly (I:C) emulsified with Montanide ISA 720 have demonstrated that the protein-based vaccine adjuvant, poly (I:C) was capable of eliciting Th1-biased adaptive immunity although the protein-based vaccine alone favors Th2-polarization. Additionally, the adjuvant potency of poly (I:C) emulsified with Montanide ISA 720 was much stronger than that of dispersed delivered poly (I:C) which suggests that protection of poly (I:C) from rapid degradation by ribonucleases was crucial for the adjuvant property of poly (I:C) [105]. Other groups have also suggested an Th1 adjuvant role of poly (I:C) [92, 139]. More importantly, induction of CD4⁺ Th cells by poly (I:C) is required for memory in CD8⁺ T lymphocytes [142]. CD8⁺ CTL memory play important roles in vaccine design for prevention of viral infection. Therefore, it seems that the adjuvant property of distinct dsRNA molecules may be different as suggested by Avril et al. [96].

Several synthetic dsRNA analogues are commercially available such as poly (I:C), poly (I:C12U), poly ICLC, poly (A:U) and PIKA. Poly (I:C) is a mismatched dsRNA with one

strand being a polymer of inosinic acid, the other a polymer of cytidylic acid. It was discovered in 1967 by Hilleman's group [143] who also discovered IFN induction by dsRNA [144] before the discovery of its molecular receptors of TLR3 [145] and MDA-5 [56, 146]. However, toxicity concerns prevent the clinical utility of poly (I:C) [147–149]. Thus, most studies using poly (I:C) as an immunoprotective or vaccine adjuvant have been undertaken in animals. Attempts to design dsRNA analogues that can induce IFN with less toxicity have resulted in poly (I:C13U) being modified to poly (I:C12U) [150]. Poly (I:C12U) differs from poly (I:C) in that every 13th cytosine of the dsRNA, cytosine (C) is replaced by uracil (U). Uracil is unable to bond hydrogen to the hypoxanthine of the partner poly (I) strand and therefore results in a mismatched base which would be more readily degraded than the parent molecule [147]. This poly (I:C) analogue has been applied in clinical trials for chronic fatigue syndrome and AIDS. It was generally well-tolerated via intravenous administration with insomnia and dry skin the most commonly reported adverse events [151]. It also exhibited profound and uniformly suppressive effects on HIV expression in vivo [152]. Poly ICLC was introduced by Levy et al. in 1975 and comprised of poly (I:C) with poly-L-lysine and carboxymethylcellulose [153]. This complex is 5–10 times as resistant to hydrolysis by primate serum as the parent poly (I:C) and has a thermal denaturation temperature about 40°C higher than that of poly (I:C). It was able to induce significant levels of serum IFN in monkey and chimpanzee under conditions in which poly (I:C) itself induced no IFN [153]. In an early clinical trial in patients with malignancy, toxic reactions composed of fever, nausea, hypotension, thrombocytopenia and leukopenia, erythema, polyarthralgia with myalgia [154]. PIKA is a stabilized dsRNA greater than 100 base pairs in length [135]. Clinical information regarding PIKA is limited and the available data are only from animals [135, 138, 155]. Stahl-Hennig et al. (2009) reported that poly (I:C) and its analogues poly ICLC and poly (I:C12U) are effective adjuvants for the induction of protein-specific cellular immune responses [104]. Among the three molecular analogues, poly ICLC was the most potent adjuvant in monkeys. This may be attributed to poly ICLC being more stable against primate serum nucleases. Another study using HIV gag as antigen showed that both poly (I:C) and poly ICLC were able to induce antigen-specific CD4⁺ T-cell response without IL-4 and IL-17 secretion, confirming Th1-polarized adjuvanticity. This adjuvant role is type I IFN-mediated [92]. Gowen et al. [156] revealed that poly (I:C) treatment significantly protected TLR3^{-/-} mice from the lethal Punta Toro virus infection despite deficiencies in cytokine induction while poly (I:C12U) was unable to protect TLR3^{-/-} mice from lethal challenge. It failed to produce IFN- α , IFN- β , and IL-6. However, in wild-type mice, poly (I:C12U) treatment was able to promote IFN- α , IFN- β , and IL-6 production and conferred protection from Punta Toro infection [156]. These results suggested that both TLR3 and MDA-5 are required for poly (I:C) to elicit immune responses but poly (I:C12U) requires only TLR3. This conclusion was verified later by Trumpfheller et al. (2008) [139]. Distinct forms of poly (I:C) with different

molecular weights or poly (I:C12U) are not equivalent in their biological behavior [96].

4.3. dsRNA as Vaccine Adjuvant against Cancer. DCs and macrophages are major sensor cells to invading pathogen and transformed cells via germ-line encoded PRRs. Upon sensing pathogens or tumor cells, activation involves these cells becoming APCs triggering innate immunity and thereby shaping adaptive immunity through cross-priming to eliminate the invading microbes and tumor cells. Cancer cells are malignantly transformed cells of the host and are capable of expressing antigens that are not expressed or in trace amount in healthy host and are referred to as tumor associated antigens (TAAs) [157]. Adaptive immunity is the major mechanism to eliminate cancer cells in the late stage of host defense responses by the generation of tumor-specific immunity [158]. Various reports have demonstrated that the recruitment of tumor infiltrating lymphocytes, especially CD8⁺ T-cells, is closely related to prognosis of the patients [159–161]. However, the anticancer immunity of the tumor-bearing host is usually weak or anergic due to either the weak antigenicity of TAAs or because of suppressive immunity in the host. Thus, enhancing the immune response, in particular TAA-specific CTL response and overcoming the immune suppression is crucial for anticancer immunity. Indeed, Provenge (also known as sipuleucel-T or APC8015) (Dendreon, Seattle, WA), a new cancer vaccine for advanced prostate cancer, was recently approved by FDA at April 29, 2010 [162]. Provenge works by ex vivo stimulation of isolated autogenous DCs of the patient with a fusion protein of full-length human prostatic acid phosphatase (PAP) and granulocyte-macrophage colony stimulating factor. This stimulation of DCs results in activation of APCs. The PAP activated APCs are suspended in lactated Ringer's solution, after removing the excessive antigen, and then infused into the patient resulting in innate and adaptive immunity against cancer cells [163]. Clinical trials have shown statistically significant prolonged median survival times [163–165]. This is the first FDA approved therapeutic tumor vaccine [166].

TLRs have been involved in the immunotherapy for cancer. Agonists of TLRs with the capacity of priming and shaping adaptive immunity have aroused significant interest in the development of cancer immunotherapy, in particular imiquimod, unmethylated cytosine preceding guanosine motif oligodeoxynucleotides (CpG ODNs), and dsRNA which act as agonistically with TLR7, TLR9, and TLR3, respectively [167].

Activation of TLR3 by dsRNA was capable of inducing either anticancer immune response or cancer cells apoptosis via TLR3 receptor expressed on a variety of cancer cells [168–173]. The two mechanisms of dsRNA against cancer work synergistically. Apoptosis of cancer cells presents the immune system with a new repertoire of TAAs in a TLR3 activation context that is favorable to the development of long-term anticancer immune responses. Poly (I:C12U) is capable of inducing phenotypic and functional maturation of DCs generated from peripheral blood monocytes of advanced ovarian cancer patients with sustained bioactive

IL-12p70 production. DCs primed with tumor lysate and matured with poly (I:C12U) are capable of generating Th1-biased specific anticancer responses in peripheral blood T-cells derived from cancer patients in the presence of ascites medium containing immunosuppressive cytokines. Using ovarian cancer ascites as an *in vitro* model, CD8⁺ T-cells derived from ascites fluid primed with tumor antigen loaded DCs matured with poly (I:C12U), exhibited cytotoxic activity with the capacity of lysis of autologous tumor cells [140, 174]. Another synthetic poly (I:C) derivative, poly ICLC, more effective as a type I IFN inducer in humans but also associated with more clinical side-effects [175], has been involved in a variety of clinical trials of malignancy treatment in the last 30 years [176–180]. When poly ICLC was used either in monotherapy or in combination therapy, it exhibited immunomodulatory effects [181] and enhancement of IL-2-induced NK lytic activity in cancer patients [179]. However, sole poly ICLC administration did not improve the survival of cancer patients [177, 179, 180]. Despite the unfavorable results of poly ICLC in tumor therapy, auspicious results were noted when used as a cancer vaccine adjuvant in mice [182]. When poly ICLC was administered with tumor antigen-derived peptide epitopes as a cancer vaccine adjuvant in a murine brain tumor model, it was capable of enhancing antigen-specific CTL response. This facilitates the infiltration of antigen-specific T-cells into the tumor site, promotes tumor homing of antigen-specific T-cells and improves the survival of tumor-bearing mice by inducing long term antitumor protection [182]. A phase I/II clinical trial using type I polarizing DCs loaded with peptides in combination with poly ICLC in patients with recurrent malignant glioma is currently being conducted [183]. Polyadenylic polyuridylic acid [poly (A:U)] is another type of synthetic dsRNA analogue that has been used in combination with chemotherapy for locally advanced gastric cancer after curative surgery patients compared with chemotherapy alone [184] despite of its inefficiency as a single adjuvant [185]. A prolonged overall and recurrence-free survival was noted. Poly (A:U) is capable of inducing Th1 cell generation and antibody production in mice when coadministered with protein [186]. *In vivo* targeted delivery of tumor associated epitope to APCs in conjunction with poly (A:U) resulted in correction of the ineffective response to idiotypic epitopes, control of tumor growth, establishment of immune memory and protection against tumors bearing antigenic variants [187]. The immunoadjuvant effects of poly (A:U) is believed to signal TLR3 and TLR7 [188].

TLR3 agonists may be an double-edged sword in cancer treatment [189]. TLR3 expresses not only on immune cells sensing dsRNA and triggering immune response but also on tumor cells exhibiting other functions [190]. It is well known that viral infection is closely related with carcinogenesis and approximately 20% of all cancers are associated with infectious agents [191] such as human papillomavirus [191] and hepatitis viruses [192]. Prevention of viral infection is able to significantly reduce the occurrence rate of cancer [193–195]. Inhibition of virus replication reduced the development of cancer dramatically even in chronically viral infected patients [194, 196]. In

this scenario, it is suspected that dsRNA, as intermediate of viral replication, is involved in the carcinogenesis. Long before the discovery of TLR3, researchers have found that dsRNA, such as poly (A:U) treatment is capable of enhancing carcinogenesis in animals [197, 198]. Studies have suspected that activation of TLRs in cancer cells could promote tumor progression and chemoresistance by activation of NF- κ B to induce upregulation of antiapoptotic proteins and to inhibit proapoptotic proteins [190]. Consistently, several groups have reported that TLR agonists stimulate the proliferation and suppressor function of Treg cells and so attenuate the antitumor effects [199, 200]. However, most studies were conducted by activation of the TLRs other than TLR3 [190, 201, 202]. Recent reports suggested that TLR3 expression is much higher in metastatic cancer cells in comparison with primary cancer cells [169]. In human hepatocellular carcinoma cells, TLR3 can be expressed both on cell surface and in cytosol and only activation of the cytoplasmic TLR3 can induce cancer apoptosis accompanied by the down-regulation of antiapoptotic protein [171]. *In situ* stimulation of TLR3 and synergistic molecule CD40 can transform ovarian cancer-infiltrating DCs from immunosuppressive to immunostimulatory cells thus exhibiting therapeutic potential of TLR3 activation [203]. Activation of TLR3 in nasopharyngeal carcinoma cells can inhibit cell migration by downregulation of chemokine receptor CXCR4 suggesting antimetastasis activity of endogenous human TLR3 expression in cancer cells [204]. Thus, it seems that activation of endogenous human TLR3 expressed by cancerous cells may induce direct pro-apoptotic activity of the tumor cells [168]. Additionally, mRNA escaping from damaged tissue or contained within endocytosed cells could serve as an endogenous ligand for TLR3 [205].

5. Perspectives

Double-stranded RNA and its synthetic analogues, such as poly (I:C) and poly (A:U) have long been known to be potent type I IFN inducers and immunomodulators [144]. However, the fact TLR3 recognized dsRNA and activates NF- κ B signal pathway was only discovered in 2001 [145]. Subsequently, other dsRNA receptors, RIG-I/MDA-5 and NLRP3 have been uncovered [7, 75, 206]. Studies of the interaction of dsRNA and its receptors have focused on immune cells. It is unknown if there are any endogenous ligands that share these receptors with exogenous dsRNA. Additionally, dsRNA receptors especially TLR3 are found expressed ubiquitously in the body. It is reasonable that not all types of cells are involved in sensing viral infection and eliciting immune response. Thus, the role of these receptors in other type of cells deserves further exploration.

dsRNA is able to induce both innate and adaptive immunity to eliminate the invading virus. However, the virus may evolve protective mechanism that enables it to destroy the dsRNA-induced signaling thereby protecting itself by evasion from the immune response of the host [207, 208]. Thus, overcoming viral protective mechanism(s) is desirable.

There is insufficient information regarding TLR3 activation in cancerous cells. Recent report suggested that

poly (I:C) binds to endo/lysosomal MDA-5 and activates apoptotic caspases in melanoma cells to induce their self degradation by autophagy and apoptosis [209]. The possibility that other dsRNA receptors may be present in tumor cells deserves further investigation. Having such knowledge would be very helpful for development of therapeutic tumor vaccine with dsRNA analogue adjuvant. In addition, the effects of dsRNA signaling on Treg cell are not known. It appears that there needs to be a balancing act between the possible carcinogenesis and the immune stimulating property of dsRNA when dsRNA analogues are considered as immunoadjuvants in tumor immunotherapy.

Although dsRNA have displayed favorable immunostimulatory and protective properties, many questions remain to be answered. Further investigations to uncover its roles in viral infection, carcinogenesis or anticancer actions deserve consideration.

Abbreviations

AP-1:	activating protein 1
ASC:	apoptosis-associated speck-like protein containing a caspase-activating and recruitment domain
CARD:	caspase recruitment domain
dsRNA:	double-stranded RNA
FADD:	Fas-associated cell death domain
IFN:	interferon
IKK:	I κ B kinase-related kinase
IL:	interleukin
IRF:	interferon regulatory factor
LRR:	leucine-rich repeat
MAVS:	mitochondrial antiviral signaling adapter
MDA-5:	melanoma differentiation-associated gene 5
NACHT:	(NAIP, CIITA, HET-E, TP-1) domain
NAIP:	Neuronal apoptosis inhibitory protein
CIITA:	Class II transactivator
HET-E:	plant Het product involved in vegetative incompatibility
TP-1:	Telomerase-associated protein 1
NAP1:	nuclear factor- κ B activating kinase-associated protein 1
NEMO:	nuclear factor- κ B essential modulator
NF- κ B:	nuclear factor- κ B
NLRP3:	nucleotide-binding domain and leucine-rich repeat containing gene family pyrin domain 3
PI3K:	phosphatidylinositol 3-kinase
PRR:	proline-rich region
PYD:	pyrin domain
RIP1:	receptor-interacting protein 1
TAB:	transforming growth factor β -activating kinase binding protein
TAK:	transforming growth factor β -activating kinase
TANK:	TRAF family member-associated NF- κ B activator
TBK1:	TRAF family member-associated NF- κ B activator-binding kinase 1
TIR:	Toll/IL-1 receptor domain
TLR3:	Toll-like receptor 3

TRADD: tumor necrosis factor receptor-associated death domain
 TRAF6: tumor necrosis factor receptor-associated factor 6
 TRIF: Toll/IL-1 receptor domain-containing adaptor inducing IFN- β .

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