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Type I interferon production by nucleic acid-stimulated dendritic cells

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1. ABSTRACT

Dendritic cells (DC) detect nucleic acid adjuvants through the Toll-like receptor (TLR) or cytosolic sensors. Nucleic acid adjuvants activate DC to produce a variety of soluble factors including proinflammatory cytokines or type I interferons (IFNs). Type I IFN, especially IFN-α, induction is a characteristic function of nucleic acid adjuvants and critical for antiviral host defense. Notably, nucleic acids derived from the host as well as from the pathogens can function as immune adjuvants and contributes to the manifestations of autoimmune diseases through the type I IFN induction. Therefore, clarifying the molecular mechanisms for type I IFN induction by nucleic acids should contribute to the development of treatment not only for viral infection but also for autoimmune diseases.

2. INTRODUCTION

DC are Ag presenting cells that can link innate and adaptive immunity (1). Immature DC reside in the peripheral tissues such as skins and capture Ags upon infection. DC also receive activation signals from the pathogens and augment the expression of costimulatory molecules and chemokine receptors such as CCR7. These maturated DC migrate into the draining lymph nodes, where DC interact with T cells and support T cell differentiation. DC activation signal can be provided by a group of receptors, called pattern recognition receptors.

Pattern recognition receptors (PRRs) that can activate DC recognize a variety of immune adjuvants (2,3). According to their ingredients, immune adjuvants can be divided into lipids, proteins, or nucleic acids. Among them,

nucleic acid adjuvants have several characteristics. First, they are recognized by PRRs in the endosome or cytosol, while lipids or proteins are detected on the plasma membrane. Second, nucleic acid adjuvants have a potent ability to induce type I interferons (IFNs) such as IFN-α or IFN-β. (4). Furthermore, host-derived nucleic acids have a similar structure to pathogen-derived ones and also behave as adjuvants. Importantly, nucleic acid-induced immune responses, especially type I IFN induction, contribute to the pathogenesis of autoimmune disorders such as systemic lupus erythematosus (SLE) (5). Thus, nucleic acid immune adjuvants play pathological as well as antiviral defensive roles. Understanding the molecular mechanisms for nucleic acid-induced adjuvant effects should pave the way for developing effective antiviral vaccines or valid treatments for autoimmune disorders. Here I review molecular mechanisms how nucleic acid sensing leads to type I IFN production and discuss pathological potentials of nucleic acid adjuvants.

3. NUCLEIC ACID-SENSING RECEPTORS

Toll-like receptor (TLRs) are representative of transmembrane PRRs (6,7). They consist of about ten family members, all of which carry a leucine-rich repeat structure in their extracellular regions. Their cytoplasmic regions are essential for signal transduction and called as the Toll/IL-1 receptor homologous (TIR) domain, based on the amino acid homology between TLR and IL-1 R families. TLR3, TLR7, TLR8 and TLR9 recognize nucleic acids and localize in the endosome (Figure1). TLR3 can recognize double-stranded RNAs (dsRNAs) (8), which are produced in virally infected cells. TLR9 can detect DNAs containing an unmethylated CpG motif (CpG DNA) (9). This motif is found more frequently in viruses or bacteria than in mammals. TLR7 was initially found to recognize synthetic chemical compounds, imidazoquinoline derivatives, that show antiviral activities (10). Several guanosine analogues are also recognized by TLR7 (11). In addition to these synthesized compounds, single-stranded RNAs (ssRNAs) from influenza virus or human immunodeficiency virus-1, which contain an uridine-rich motif, function as natural TLR7 agonists (12,13). TLR8 is very close to TLR7 in terms of the amino acid structure and chromosomal location. TLR7 and TLR8 genes are only about 50 kbs apart in X chromosome. An imidazoquinoline derivative, R848 can activate murine TLR7 as well as human TLR7 and TLR8, but not murine TLR8, indicating that murine TLR8 is non-functional. However, it was also reported that murine TLR8 is involved in the activity of polyT-containing oligodeoxynucleotides (ODNs) to augment the effect of imidazoquinoline derivatives (14,15) .

The retinoic acid-inducible gene I (RIG-I)-like receptors (RLRs) function as nucleic acid sensors in the cytosol (Figure1). RLRs include RIG-I, melanoma differentiation associated gene 5 (MDA5) and LGP2 (16). All of them carry an RNA helicase domain and RIG-I and MDA5 possess a caspase recruitment domain (CARD) at their N-terminals. Both RIG-I and MDA5 can bind dsRNA *in vitro*, which makes their specific roles unclear. However, gene targeting experiments clarified their differential roles *in vivo*. RIG-I is essential for responses to Sendai virus, Newcastle disease virus, influenza A virus and Japanese encephalitis virus, whereas MDA5 is critical for detecting picornaviruses including encephalomyocarditis virus (EMCV) (17). Furthermore, MDA5, but not RIG-I, is essential for responses to synthetic dsRNA mimic, polyinosinic-polycytidylic acid (poly (I:C)). The target structure of RIG-I is a free triphosphate at the 5' end of RNA, which is modified by capping in the host (18,19). The structure does not depend on ssRNAs or dsRNAs and is found in the genomes of influenza virus, but not of picornavirus, which is consistent with the results of gene targeting mice. The structures recognized by MDA5 are still unknown. LGP2 lacks a CARD domain and functions *in vitro* as a negative regulator (20). However, LGP2-deficient mice showed impaired responses against ECMV, but enhanced responses against vesicular stomatitis virus or poly (I:C), indicating certain positive roles in viral infection (21). It also remains unknown how LGP2 recognize viral-derived components.

Double-stranded DNAs (DsDNAs) also function as an immune adjuvant and recognized in the cytosol. Its adjuvanticity depends on the right-handed helical B form, which is widely found in nature (22). A cytosolic protein, DNA-dependent activator of IRFs (DAI), previously named DLM-1 or Z-DNA binding protein 1, was reported to be involved in this recognition. DAI can bind dsDNA and overexpressed DAI can enhance the ability of dsDNA to induce type I IFNs. Furthermore, knockdown of DAI leads to impaired induction of type I IFNs by dsDNA and a DNA virus, herpes simplex virus-1 (23).

4. TLR7/9 AND TYPE I IFN

DC are heterogeneous and consist of several subsets (24). DC respond to nucleic acids in a subset-specific manner. In the murine spleen, DC can be divided into two groups, plasmacytoid DC (pDC) and conventional DC (cDC). Murine BM-derived DC can be generated by culturing BM cells in the presence of Flt3L and Flt3L-induced BM DC also include both pDC and cDC. Both pDC and cDC express CD11c, while pDC express several PDC-specific markers, including B220, mPDCA-1, SiglecH, and Ly49Q, all of which are not expressed in cDC. pDC express TLR7 and TLR9 exclusively among TLRs and produces vast amounts of type I IFN including IFN-α and IFN-β (25). cDC can also produce IFN-β in response to TLR7/9 signaling, but fail to produce IFN-α in response to these TLR agonists. Therefore, pDC are featured by the ability to produce IFN-α in response to TLR7/9 signaling. Cytosolic nucleic acid sensors do not play major roles in pDC (26), indicating that pDC can be regarded as a type I IFN producing DC subset by sensing nucleic acids through TLR7 and TLR9. Type I IFNs derived from TLR7/9-stimulated pDC can activate NK or T cells and induce B cell maturation, thereby contributing to antiviral host defense (25). Furthermore, as described below (see section 7), TLR7/9-induced type I IFN induction is also critical for the pathogenesis of autoimmune disorders. Thus, it is very important to clarify the molecular mechanisms by which

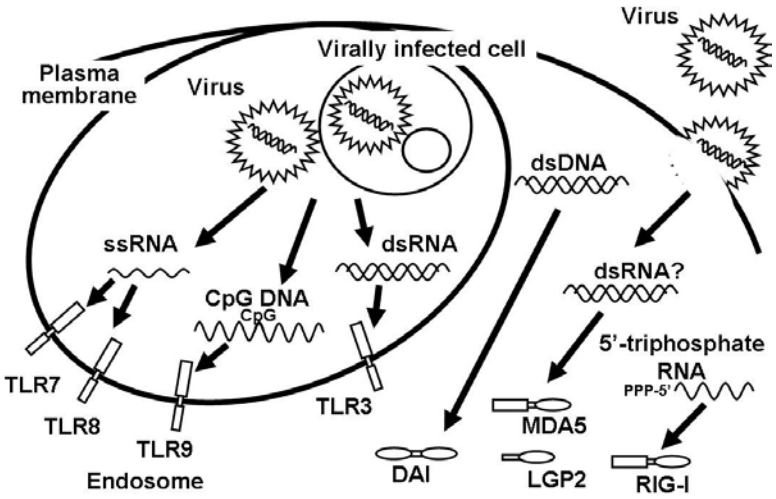

Figure 1. Nucleic acid recognition by PRRs. TLRs are expressed in the endosome and detect nucleic acids from virus or virally infected cells. RNA helicases and DAI localize in the cytosol.

TLR7/9 signaling leads to type I IFN induction, especially in pDC.

TLR7 and TLR9 can activate similar signal transduction pathways. A cytoplasmic adapter, MyD88, which can associate with TLR family members except TLR3, is essential for TLR7/9 signaling (Figure 2). MyD88-deficient DC are refractory to TLR7/9 agonists and fail to produce either type I IFNs or proinflammatory cytokines including IL-12 or TNF. At the downstream of MyD88, the signaling pathway is bifurcated. One pathway requires NF-κB activation, which can lead to production of proinflammatory cytokines. This pathway is called as a classical or canonical NF-κB activation pathway (27). This NF-κB activation depends on phosphorylation and degradation of IκB, which retains NF-κB as an inactive form in a cytosol. A serine threonine kinase, IκB kinase (IKK) family includes four members, IKKα, IKKβ, TANK-binding kinase 1 (TBK1) and inducible IKK (IKKι) (also known as IKKε, hereafter shown as IKKε/ι) (27,28). IKKβ is critical for this canonical NF-κB activation. The other pathway requires interferon regulatory factor (IRF)-7 activation, which can lead to production of type I IFNs (29). This type I IFN induction requires IRF-7 to be phosphorylated and translocated into the nucleus. IRF-7 phosphorylation and activation depend on another IKK family member, IKKα (30). In addition, a serine threonine kinase, IRAK-1, was also reported to be involved in IRF-7 phosphorylation (31), although it is unclear how IKKα and IRAK-1 interact to activate IRF-7.

Analysis on a series of CpG oligonucleotides (ODNs) revealed that certain types of CpG ODNs have a high ability to induce type I IFNs (32). Such CpG ODNs carry oligo-dG tail at their 3' terminal and this facilitates formation of higher order structures through the G-tetrad formation (33). Then CpG ODNs form nucleic acid-based nanoparticles, which are similar to viruses in size and can be retained for long periods in the early endosome of pDC (34,35). The MyD88-IRF-7 complex is rich in the endosome. In contrast, in cDC, the CpG ODNs are rapidly transported and degraded in the lysosome. Importantly, when conjugated with certain types of lipids that can retain CpG ODNs in the early endosome, the CpG ODNs can activate cDC to produce type I IFNs. Thus, type I IFN induction by TLR9 signaling depends on an intracellular fate of the agonists (34,35).

Several other molecules are also reported to be involved in TLR7/9-induced type I IFN production in pDC. A phosphoprotein, osteopontin, was previously considered to be a kind of cytokine to be secreted, but suggested to function as a MyD88-interacting intracellular molecule critical for type I IFN induction by TLR7/9 (36). Furthermore, another adapter, TRAF3, is a major regulator for type I induction by a variety of innate stimuli including TLR7/9 (37,38). Further analysis is required to clarify how these molecules cooperate with MyD88, IKKα, and IRAK-1.

5. TLR3/4 AND TYPE I IFN

TLR3 and TLR4 are involved in recognizing dsRNA and LPS, respectively. These TLRs are distinct from the other TLRs in that they can activate the MyD88-independent pathway, which is mediated by another cytoplasmic adapter, TRIF, possessing a TIR domain (Figure 3) (39,40). TLR4 signaling depends on both MyD88 and TRIF, while TLR3 signaling depends on TRIF, but not on MyD88. At the downstream of TRIF, IRF-3 is phosphorylated, forms a homodimer and translocates into the nucleus, where it activates the promotor of the IFN-β gene.

Among IKK family members, TBK1 and IKKε/ι are more similar to each other in amino acid structures than to IKKα or IKKβ. TBK1 and IKKε/ι fail to fully phosphorylate IκB and IκB is not degraded by TBK1 or

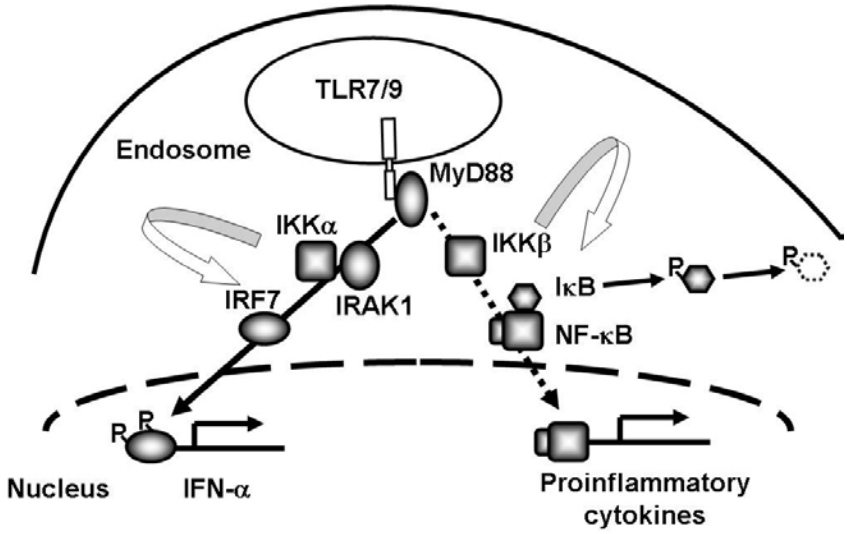

Figure 2. Signaling pathways for TLR7 and TLR9. TLR7 and TLR9 can activate similar signaling molecules, thereby leading to production of proinflammatory cytokines and type I IFNs. MyD88 is an essential adapter for both pathways. In activating NF-κB, IKKβ works as a heterodimer with IKKα, but an IKKβ homodimer can compensate the function of the heterodimer in the absence of IKKα. In activating IRF-7, IKKα and IRAK1 are required. Phosphorylation is indicated by tortuous arrows.

IKKε/ι. Furthermore, TBK1 and/or IKKε/ι deficiency does not lead to defective NF-κB activation. Thus, these kinases are not essential for NF-κB activation. These kinases, however, are involved in phosphorylation and activation of IRF-3 (Figure 3) (41,42). IRF-3 activation and subsequent IFN-β induction in response to TLR3/4 signaling were impaired in TBK1-, but not in IKKε/ι-, deficient cells, indicating that TBK1 plays dominant roles *in vivo* (43,44). However, TBK1 can form a heterodimer with IKKει and analysis on the mutant mice lacking both TBK1 and IKKει demonstrated that IKKει also functions cooperatively with TBK1.

6. CYTOSOLIC SENSORS AND TYPE I IFN

Cytosolic sensors such as RIG-I and MDA5 associate through a CARD domain with a cytoplasmic adapter molecule, IFN-β promoter stimulator 1 (IPS-1), also called as mitochondrial antiviral signaling protein (MAVS), virus-induced signaling adapter (VISA), or CARD adaptor inducing IFN-β (CARDIF) (45-48) and leads to activation of IRF-3 and IRF-7 and type I IFN gene expression. This activation of IRFs depend on TBK1 and IKKε/ι DAI-induced type I IFN induction also requires TBK1 and IRF-3 (23). However, IPS-1 is not essential for this pathway (49).

Notably, cytosolic sensor-induced pathway can lead to IFN-α production, while TLR4 signaling cannot. Furthermore, there are no definitive evidences that TLR3 signaling can induce IFN-α production. Thus, TLR3 and TLR4 fail to induce IFN-α production, although they can activate TBK1 and IKKε/ι. It can be assumed that cytosolic sensor signaling can induce IFN-α by activating the additional pathway or modulating TBK1 and IKKε/ι in a cytosolic sensor-specific manner.

7. TLR7/9 IN AUTOIMMUNITY

Lipid ligands including LPS or various types of lipoproteins are bacterial cell wall components. A protein ligand, flagellin, is a TLR5 ligand and a constituent for a flagella. Therefore, these structures are definitely nonself for the mammals. However, nucleic acid TLR ligands such as CpG DNA or ssRNAs are not strictly distinct from host-derived nucleic acids. Unmethylated CpG motifs can be found in the mammals, albeit at a low frequency. Furthermore, host-derived mRNA or mRNA encoding green fluorescence protein can function as TLR7 ligands (12). In this context, self-derived nucleic acids have the potential to sitmulate TLR7/9-signaling, which can lead to autoimmune reactions. In other words, nucleic acid-sensing system by TLR7/9 is vulnerable to autoimmunity.

This danger is prevented by several fail-safe mechanisms. First, nucleic acids are quite unstable due to their sensitivity to nucleases. Second, self nucleic acids and TLRs localize separately within the cell. Self RNA is present in the cytoplasm and self DNA is localized mainly in the nucleus. Thus, self nucleic acids normally fail to translocate into the endosomal compartment, where TLR7 and TLR9 are expressed. In contrast, virus-derived nucleic acids can be accessible to the endosome after viruses or virally infected cells are endocytosed (Figure 1). Furthermore, the fact that TLR9 is not expressed in the plasma membrane is also important. Engineered expression of TLR9 on the plasma membrane makes TLR9 responsive to self DNA (50). Thus, endosomal expression of TLR7 and TLR9 is advantageous for the host to avoid self-recognition and provoke antiviral immune responses.

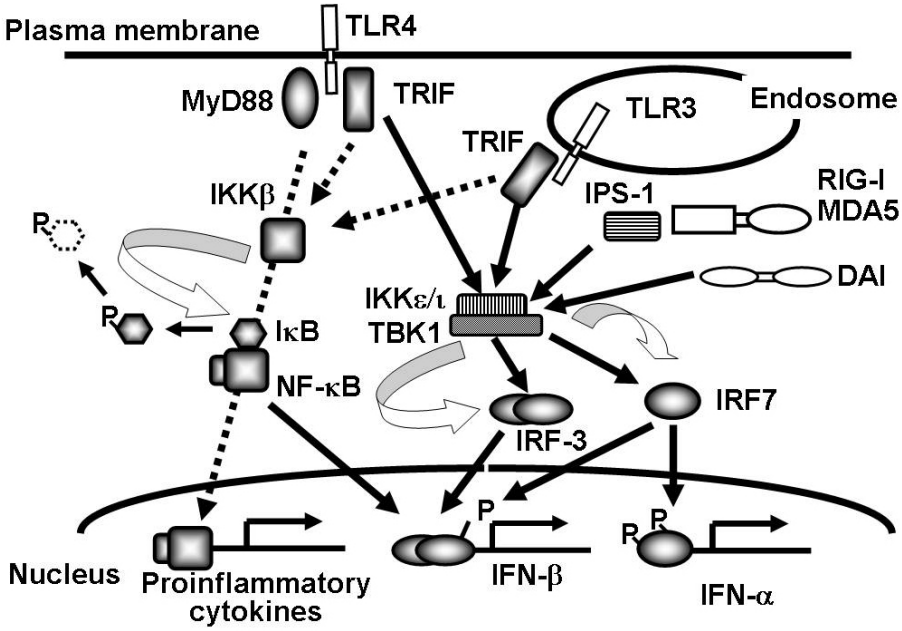

Figure 3. Signaling pathways for TLR3/4 and cytosolic sensors. TLR3 and TLR4 can induce IFN-β production in a TRIF-dependent manner. At the downstream of TRIF, TBK1 and IKKε/ι play critical roles as a heterodimer and can lead to IRF-3 activation. TLR3 and TLR4 can also activate classical NF-κB activation pathway, which is involved in optimal IFN-β gene induction. Cytosolic sensors such as RIG-I and MDA5 can activate IRF-3 and IRF-7 through a heterodimer of TBK1 and IKKε/ι in an IPS-1-dependent manner. DAI can also activate IRF-3 and IRF-7 through TBK1, but this pathway does not require IPS-1. Notably, TLR3/4 signaling fails to induce IFN-α gene expression. Phosphorylation is indicated by tortuous arrows.

TLR expression pattern among DC subsets also contributes to prevention of self-recognition. TLR7 expression is lowest in a $CD8^+$ DC subset, which has high phagocytic activity (51). Thus, self RNA derived from incorporated cells has little chance to act through TLR7. It is also notable that certain regions in the host DNA can inhibit TLR9 signaling. Even at high doses, mammalian DNA containing an unmethylated CpG motif cannot activate B cells, indicating that high frequency of a CpG motif is not a sole factor for TLR9 activity of microbial DNA (52). Several types of DNA sequences that can inhibit TLR9 signaling have been identified (53) and some of them can be found in the telomere (54). Furthermore, host-derived RNAs are distinct from virus-derived RNAs in that it has modified nucleosides, which include 5-methylcytidine, N6-methyladenosine or pseudouridine. This modification significantly reduces immunostimulatory activity (55,56).

Thus, with several strategies the host tries to avoid nucleic acid-induced immune reactions. This homeostatic balance can be broken by several mechanisms (Figure 4). In autoimmune disorders such as SLE, autoAbs aganinst DNA, RNA or ribonuclear proteins (RNPs) including DNA or RNA are generated. These Abs bind nucleic acids and form immune complexes (ICs). Nucleic acids in ICs are rich in unmethylated CpG DNAs and uridine-rich RNAs, which can function as TLR9 and TLR7 ligands, respectively (57). These ICs are more stable than free nucleic acids and can be accessible to DC through FcγRIIa (58). Then they meet their sensors, TLR7 or TLR9, in the endosome.

Endogeneous molecules are also involved in facilitating the TLR9 signaling. High-mobility group box 1 (HMGB1) is a nuclear protein that can bind DNA and regulate gene expression. HMGB1 is actively secreted from cells by stimulation with proinflammatory cytokines or released from necrotic cells, which can be generated upon tissue injury (Figure 4). HMGB1 is an essential component of DNA-containing ICs for triggering TLR9 signaling (59). This function is also dependent on an immunoglobulin superfamily member, receptor for advanced glycation end-products (RAGE), that can bind HMGB1 (59).

Psoriasis is an autoimmune skin disorder in which autoimmune T cells are activated and induce abnormal differentiation of epidermal keratinocytes (60). Lande *et al.* have found that psoriatic skin lesions contained type I IFN inducing activity and that one active fraction contained an antimicrobial peptide, LL37 (61). Expression of LL37 was elevated in psoaritic skin lesions, but not in the normal skin or skin lesions of atopic dermatitis. Interestingly, the IFN inducing activity was dependent not only on LL37 but also on DNAs. LL37 forms a complex with DNA, is incorporated by pDC and is retained in the endosome, where it stimulates TLR9 signaling (Figure 4). Thus, endogeneous molecules including nuclear proteins or antimicrobial peptides can facilitate TLR9-induced immune responses.

Type I IFNs induced by TLR7/9 signaling are important for establishing the manifestations of autoimmune disorders (62). During the IFN-α therapy,

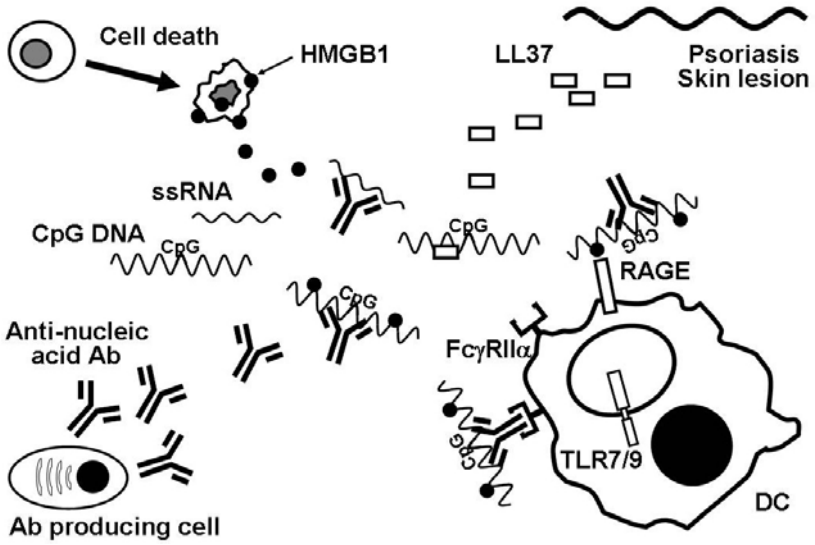

Figure 4. Molecular mechanisms for facilitating the TLR7/9 function. Usually host-derived nucleic acids have little chances to meet TLR7 and TLR9, which are expressed in the endosome of DC. Several molecules can contribute to the accession of nucleic acids to the TLRs. Anti-nucleic acid Abs bind and stabilize host-derived nucleic acids. Then ICs activate FcγRIIa signaling or are incorporated by FcγRIIa into DC. Upon cell death, nuclear proteins including HMGB-1 are released and facilitate the uptake of nucleic acids by DC. HMGB-1 can also activate its receptor, RAGE. In the skin lesions from psoriasis, an antimicrobial peptide, LL37, is overproduced and transports DNAs to the endosome in DC.

autoAb production was observed in cancer patients. Serum type I IFN concentrations are elevated in SLE patients and the patient-derived peripheral mononuclear cells show IFN signature, which is characterized by increased expression of type I IFNs and IFN-inducible genes (63). In SLE or psoriasis, a type I IFN producing DC, pDC, are activated and increased in the skin lesion (64,65).

Type I IFNs can upregulate expression of MHC genes and induce DC maturation. They can also stimulate cross-priming ability of DC (66). Cross-priming is an ability of DC to present exogeneous Ags with MHC class I for generating cytotoxic $CD8^+$ T cells (67). Such Ags can be derived not only from virally infected cells or tumor cells but also from apoptotic or necrotic host-derived cells. In addition to DC, B cells are also activated by type I IFNs to differentiate into plasma cells that can produce autoAbs (63). These various immune stimulating functions of type I IFNs should contribute to the manifestations of autoimmune diseases.

Murine autoimmune models also implicate critical roles of TLR7. BXSB mice manifest autoimmune nephritis which depends on the abnormal Y chromosome (68). This Y chromosome is called Yaa, which represents Y-linked autoimmune accelerator and carries duplication of genomic DNA regions including the *TLR7* gene (69,70). In these mutant mice, B cells produce several types of autoAbs and become hyperreactive to TLR7 signaling. Increase of TLR7 expression level is sufficient enough to cause the manifestation (71). Interestingly, in lupus-prone $MRL/Mp^{lpr/lpr}$ mice, production of autoAbs and pathological findings were ameliorated in TLR7 deficiency, but exaggerated in TLR9 deficiency (72). *In vitro* findings so far have not clearly distinguished the function of TLR7 from that of TLR9. Therefore, the results indicate the possibility that TLR7 and TLR9 play distinct roles in regulating autoimmune responses *in vivo*.

8. CONCLUSION

Nucleic acids are characterized by their type I IFN-inducing ability. Importantly endogenous molecules behave also as the agonists or facilitators for TLR7/9 agonists and contribute to the deterioration of autoimmune disorders. Signaling pathways for type I IFN production have been progressively revealed, but the detailed mechanisms are still unclear and we have not got effective maneuvers for manipulating the type I IFN production. We should clarify the molecular mechanisms and identify the signaling molecules which should be targeted for reinforcing antiviral defense mechanisms or treating the autoimmune diseases.

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Abbreviations: PRR: Pattern recognition receptor; DC: dendritic cell; TLR: Toll-like receptor; IFN: interferon; SLE: systemic lupus erythematosus; TIR: Toll/IL-1 receptor homologous; dsRNA: double-stranded RNA; CpG DNA: DNAs containing an unmethylated CpG motif; ssRNA: single-stranded RNA; RIG-I: retinoic acid-inducible gene I; MDA5: melanoma differentiation associated gene 5; CARD: caspase recruitment domain; EMCV: encephalomyocarditis virus; poly (I:C); polyinosinic-polycytidylic acid; dsDNA: double-stranded DNA, DAI: DNA-dependent activator of IRFs; pDC: plasmacytoid DC; cDC: conventional DC; IKK: IκB kinase; TBK1: TANK-binding kinase 1; IKKι: inducible IKK; IRF: interferon regulatory factor; ODN: oligodeoxynucleotide; IPS-1: IFN-β promoter stimulator 1; MAVS: mitochondrial antiviral signaling protein; VISA: virus-induced signaling adapter; CARDIF: CARD adaptor inducing IFN-β; RNP: ribonuclear protein; HMGB-1: High-mobility group box 1; RAGE: receptor for advanced glycation end-products

Key Words: Dendritic cells, Type I IFN, Toll-like receptor, nucleic acid, adjuvant, RIG-I, IRF, SLE, psoriasis, Autoimmunity, pDC, cDC, IKK, NF-kappaB, Signaling, Review

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