Chapter 12
Immune Recognition of Nucleic Acids and Their Metabolites

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Y. Kikuchi and E. Rykova (eds.), *Extracellular Nucleic Acids*,
Nucleic Acids and Molecular Biology 25,
DOI 10.1007/978-3-642-12617-8_12, © Springer-Verlag Berlin Heidelberg 2010

Abstract Recent research suggests that nucleic acids are active modulators of the immune system. RNA and DNA can be detected by specific receptors – the so-called Toll-like receptors, RIG-I-like receptors, and NOD-like receptors. Resultant intra- and intercellular activations of the innate immune system are pivotal in both protective and pathological immune responses during infection and other immunological disorders. Moreover, our immune system is substantially modulated by metabolic intermediates of nucleic acids. Elucidation of such manifold mechanisms involved in immune recognition of nucleic acids and their metabolites offers the possibility of novel nucleic acid-based immunotherapies as well as interventions for nucleic acid-related immune disorders.

12.1 Introduction

Nucleic acids of mammalian cells have long been thought to be immunologically inert as they are normally tightly packed and sequestered within the nucleus. However, recent progress in immunology has shown that nucleic acids and their metabolites are specifically detected by the innate immune receptors, also called Pattern Recognition Receptors (PRRs); these include Toll-like receptors (TLRs), Retinoic Acid Inducible Gene-I (RIG-I)-like Receptors (RLRs), and Nod-like Receptors (NLRs). These receptors are able to distinguish "non-self," such as infectious organisms or damaged host cells, from "self" moieties of host or environmental entities, activating intra- and intercellular signaling pathways of the immune system through receptor-mediated recognition of dangerous "non-self" signatures.

Here, we show how the host immune system recognizes and responds to "non-self" nucleic acids in abnormal conditions, such as microbial infections as well as to "self" nucleic acids in certain immunological disorders, such as autoimmune diseases. We also illustrate some clinical applications of nucleic acids that utilize their immunogenic potential, such as vaccine adjuvants.

12.2 The Fundamental Mechanism to Avoid False Recognition of Harmless Nucleic Acids: The Case of Food Metabolism

Metabolizing food enables us to generate energy through ATP and acetyl CoA production. It also allows us to synthesize nucleic acids for cell proliferation (de novo pathway) and to recycle nucleic acids within food metabolites (salvage pathway). Nucleic acids in foods are metabolized from polynucleotides to nucleosides to free bases, using several kinds of digestive enzymes, including endonucleases, phosphodiesterases, and nucleoside phosphorylases. For instance, in the case of purine nucleotide metabolism, phosphate and ribose are successively separated from purine bodies in food by the above enzymes in the small intestine,

yielding free bases like guanine and hypoxanthine (Ishii and Akira 2008). Free bases can be absorbed for recycling through special transporters on cell surface such as Nucleobase-Cation-Symport-1 (NCS1), the structure of which was recently published (Weyand et al. 2008). Unnecessary bases are degraded to uric acids and eliminated to urine, via xanthine. Neither polynucleotides nor nucleosides can enter cytoplasm unless they are degraded to free bases. While this mechanism seems inefficient, it is a fundamental means of avoiding misidentification of harmless or self nucleic acids inside host cells (Ishii and Akira 2008).

12.3 The Mechanism to Distinguish Healthy "Self" Nucleic Acids

Recent research has shown several unique receptors to recognize nucleic acids and their metabolites (Table 12.1; Figs. 12.1 and 12.2). Some of these receptors are expressed on cell surfaces and some are in cytosol or on endosomal membranes. Some of these receptors are specialized to recognize Pathogen-Associated Molecular Patterns (PAMPs); others detect Danger-Associated Molecular Patterns (DAMPs), which are endogenous products released from damaged host cells (Matzinger 2002; Kono and Rock 2008).

Here, we review how the immune system senses and responds to exogenous detrimental nucleic acids adequately in the case of microbial infections (3.1), controls reactions to endogenous nucleic acids in the case of host cell damage (3.2; 3.3), and misidentifies endogenous nucleic acids in the case of autoimmune diseases (3.4).

Table 12.1 Pattern recognition receptors for nucleic acids and their metabolites

Receptor family	Location	Major ligands	Receptor adaptor
Toll-like receptors (TLRs)	Endosome	ssRNA	TLR7/8 MyD88
		dsRNA	TLR3 TRIF
		Unmethylated CpG in ssDNA, abnormal DNA	TLR9 MyD88
RIG-I-like receptors (RLRs)	Cytosol	5′-triphosphate ssRNA	RIG-I IPS-1
		dsRNA	MDA5 IPS-1
		dsRNA	LGP2 IPS-1
NOD-like receptors (NLRs)	Cytosol	Microbial and synthetic RNA	NLRP3 ASC
Purinergic receptors	Cell surface	Adenosine	P1 None
		Nucleotides (ATP, UTP, ADP, UDP)	P2X None
		ATP	P2Y None
Others	Cytosol	dsDNA	AIM2 ASC
		dsDNA	DAI Unknown

MyD88 myeloid differentiation primary response gene 88, *TRIF* TLR-domain-containing adapter-inducing interferon-β, *RIG-I* retinoic acid-induced gene I, *IPS-1* interferon-β promoter stimulator-1, *MDA5* melanoma differentiation-associated gene-5, *ASC* apoptosis-associated speck-like protein containing a CARD, *AIM2* absent in melanoma 2

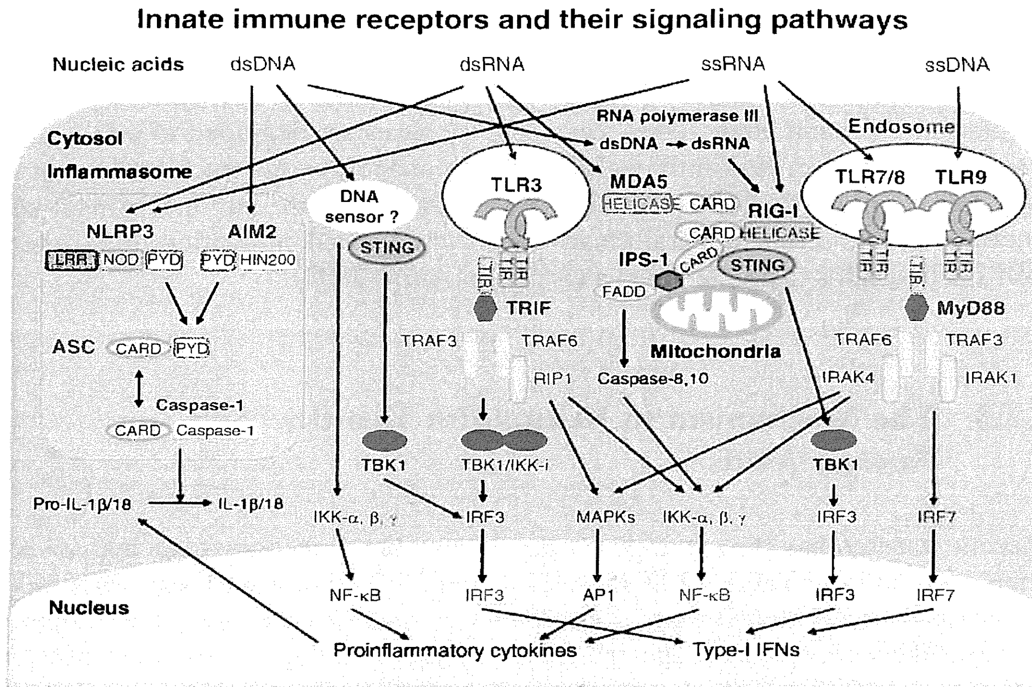

Fig. 12.1 ssRNA and dsRNA are detected by TLR7/8, RIG-I, NLRP3, and TLR3, MDA5, NLRP3, respectively. ssDNA and dsDNA are detected by TLR9 and TBK-dependent unknown DNA sensors, AIM2, RNA polymerase III, respectively. TLR3, TLR7/8, and TLR9 are located in endosomes. TLR3 signals through TRIF. TRIF associates with TRAF3, TRAF6, and RIP1. TRAF3 activates IRF3 via TBK1/ IKK-i, whereas TRAF6 and RIP1 activate MAPKs and NF-κB. TLR7/8 and TLR9 signal through MyD88. MyD88 also associates with TRAF3 and TRAF6. They form a complex with IRAK-4, IRAK-1, and activate IRF7, NF-κB, and MAPKs. RIG-I, MDA5, NLRP3, and AIM2 are located in the cytoplasm. Both RIG-I and MDA5 signal through IPS-1, which interacts with STING and FADD. STING activates IRF3 via TBK1 and FADD activates NF-κB through cleavage of caspase-8/10. NLRP3 and AIM2 associate with ASC. ASC-dependent inflammasome activation induces IL-1β /18 production via caspase-1. RNA polymerase III converts dsDNA into dsRNA and such dsRNA detected by RIG-I. *TBK1* TANK-binding kinase 1, *TRAF* TNF receptor-associated factor, *RIP* receptor-interacting protein, *IRF* Interferon regulatory factor, *IKK* IκB kinase, *NFκB* nuclear factor-kappa B, *MAPKs* mitogen-activated protein kinases, *IRAK* IL-1R-associated kinase; *NFκB* nuclear factor-kappa B; *MAPKs* mitogen-activated protein kinases; primary response gene 88, *STING* stimulator of interferon genes, *FADD* Fas-associated death domain

12.3.1 The Mechanism to Detect Exogenous Nucleic Acids Breaking into Host Cells: The Case of Microbial Infections

The immune response is the host's first-line protection from pathogens. In the case of microbial infections, nucleic acids can be released from microorganisms and infected host cells simultaneously. Therefore, the host has evolved a specialized mechanism to discriminate whether these nucleic acids should be eliminated or ignored, based on their sequences, structures, or modifications (Akira et al. 2006;

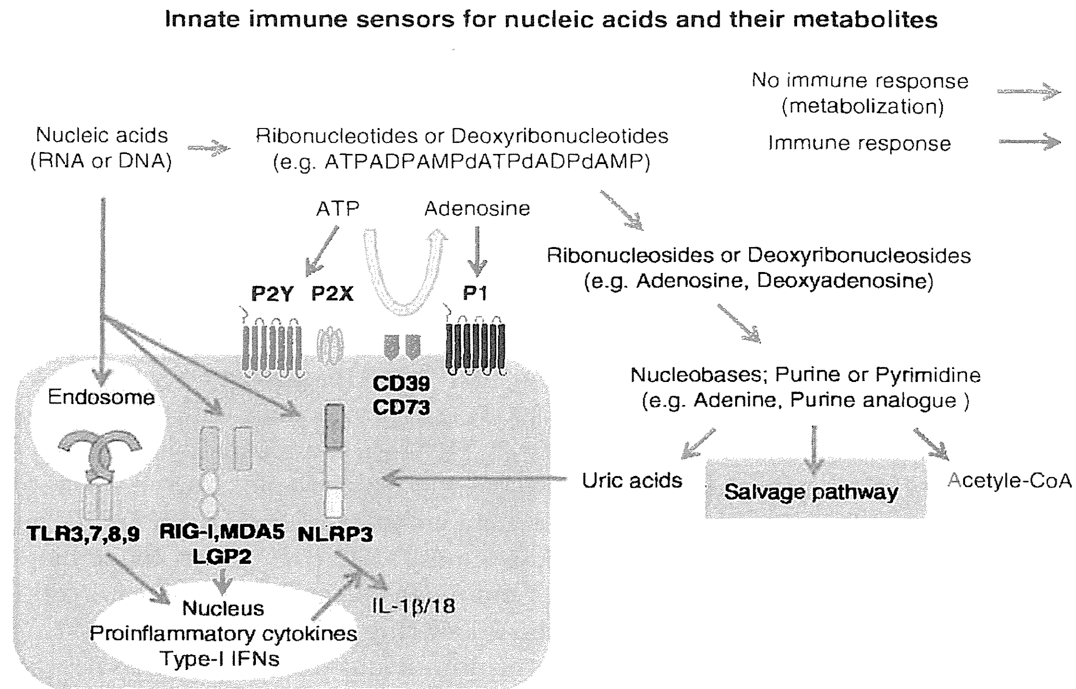

Fig. 12.2 Metabolization (*blue arrow*): Nucleic acids in foods are metabolized from polynucleotides to nucleosides to free bases, using several kinds of digestive enzyme. Free bases can be used for acetyl CoA production (energy) or recycling (salvage pathway) or degraded to uric acids and eliminated to urine. Immune response (red arrow): Nucleic acids and their metabolites can be released from damaged or dying host cells in the case of infection or tissue injury. For example, viral RNA or DNA can be directly detected by endosomal TLRs and cytoplasimic RLRs and NLRs. In addition, aberrant high concentration of extracellular nucleotides incuding ATP, adenosine, and their metabolic end products, uric acids, can be detected by innate immune sensors containing NLRs, ATP-gated P2 receptor, and G protein-coupled P1 receptors as danger signals. These responses mainly culminate in IL-1β/18 production via inflammasome activation. Adenosine is degraded from ATP through a cascade of ectonucleotidases, including nucleoside triphosphate diphosphorylase (NTPDase, also called CD39) and 5′-ectonucleotidase (Ecto50NTase, also called CD73)

Ishii et al. 2008b). Here, we illustrate several systems involved in the recognition of exogenous nucleic acids through immune receptors, including TLRs, RLRs, and NLRs.

12.3.1.1 Endosomal Recognition for Exogenous Nucleic Acids via TLRs

Currently, 11 members of the TLR family have been identified as functional receptors in mammals. Among them, TLR-3, -7, -8, and -9 have been shown to recognize nucleic acids within early endosomes (Takeda and Akira 2005). TLRs have ectodomains that contain variable numbers of Leucine-Rich-Repeat (LRR) motifs and a cytoplasmic signaling domain termed the Toll/IL-1R homology (TIR) domain. LRR motifs are responsible for sensing nucleic acids; the crystal structures

of TLR-3 (but not TLR-7, -8 or -9) have been shown to have horseshoe-like solenoid shapes (Choe et al. 2005; Leonard et al. 2008). TLR-3 recognizes double-stranded (ds) RNAs, which can be intermediates for single-stranded (ss) RNA viral replication, symmetrical transcription byproducts of DNA viruses, or synthetic compounds (e.g., poly IC) (Alexopoulou et al. 2001). TLR-3 is expressed in immune cells, including Dendritic Cells (DCs) and macrophages; it is also expressed in nonimmune cells such as epithelial cells, and it is inducible through type-I IFN response (Alexopoulou et al. 2001; Tissari et al. 2005). Double-stranded RNA recognition via TLR-3 can occur when virus-infected cells are phagocytosed by immune cells (Schulz et al. 2005). TLR-3 signals through the adaptor molecule TIR domain containing adaptor inducing IFN-β (TRIF, also known as TICAM1) (Yamamoto et al. 2003; Oshiumi et al. 2003). TRIF associates with tumor necrosis factor-receptor associated factor (TRAF)-3, TRAF-6, and receptor-interacting protein (RIP)-1. This complex activates NF-κB via IκK-complex, AP-1 via MAPK, and Interferon Response Factor (IRF)-3 via a complex of the TRAF family member-associated NK-κB activator-Binding Kinase 1 (TBK1) with Inducible IκB Kinase (IκKi), initiating pathways that culminate in proinflammatory cytokines and IFN-β production (Akira and Takeda 2004) (Fig. 12.1).

TLR-7 and -8 recognize uridine-rich or uridine/guanosine-rich ssRNA of virus (e.g., influenza virus, human immunodeficiency virus) and synthetic compounds (e.g., imidazoquinolines) (Diebold et al. 2004; Jurk et al. 2002). The expression of TLR-7 is limited to B cells and DCs in human and mice, while TLR-8 functions primarily in human monocytes and myeloid DCs (Hornung et al. 2008). Both genes are located on the X chromosome. TLR-9 preferentially recognizes unmethylated CpG motifs (lack of cytocine methylation) in microbial DNA, which is thus discernable from highly methylated CpG motifs in mammalian DNA (Hemmi et al. 2000; Krieg 2002; Wagner 2004; Klinman 2004); TLR-9 binding to its cognate ligand induces a conformational change that activates the downstream intracellular signaling pathway (Latz et al. 2007). Sequence restriction of TLR-9 recognition varies as unmethylated CpG motifs are needed to activate TLR-9 only in ssDNA with phosphorothionate backbones, whereas natural phosphodiester DNA can activate TLR-9 independently of CpG motifs (Haas et al. 2008). In addition, TLR-9 activation by synthetic oligonucleotides (ODNs) is dependent on cell types (Ishii et al. 2004) and secondary structures of ODNs, such as aggregated forms (Kerkmann et al. 2005; Hou et al. 2008). The expression of TLR-9 is restricted to Plasmacytoid DCs (pDCs) and B cells in humans, while expression of mouse TLR-9 is broader, as TLR-9 is highly expressed in murine myeloid DCs and macrophages (Kadowaki et al. 2001; Bauer et al. 2001; Hornung et al. 2002).

The proteins TLR-7, -8, and -9 share a common signaling pathway through a TIR domain – containing adaptor molecule, Myeloid Differentiation Factor 88 (MyD88). MyD88 associates with TRAF3/6 and interleukin-1-receptor-associated kinase (IRAK)-1/4. This complex activates NF-κB, AP-1, and IRF7 via IκK-complex, MAPK and IκKα, respectively, and culminates in proinflammatory cytokines and IFN-α/β production (Akira and Takeda 2004). IRF7 is constitutively expressed in pDCs and is especially involved in inducing massive type-I IFNs response via

the TLR-7/9-MyD88-dependent signaling pathway (Kawai and Akira 2006) (Fig. 12.1).

Notably, TLR activation upon recognition by nucleic acids occurs mainly in the endosome. Conceivably, TLR localization in the endosome is necessary to prevent contact with "self" nucleic acids, which are not taken into the endosome without additional components, as described below.

12.3.1.2 Cytoplasmic Sensors for Exogenous Nucleic Acids

Although endosomal TLRs that recognize nucleic acids are expressed mainly in the specialized immune cells, such as B cells and DCs, there are receptors in the cytoplasm that are also capable of sensing nucleic acids, namely RLRs and NLRs. In addition, several molecules have been proposed as capable of recognizing double-stranded B-form DNA (Takeshita and Ishii 2008; Vilaysane and Muruve 2009).

RIG-I and Melanoma Differentiation-Associated gene 5 (MDA5) belong to RLRs (Yoneyama et al. 2004; Takeuchi and Akira. 2008). Both of these cytoplasmic proteins contain helicase domains, including ATP-binding domains, C-terminal regulatory domains capable of binding tRNA, and Caspase Activation and Recruitment Domains (CARDs) to interact with adaptor molecule Interferon-β Promoter Stimulator 1 (IPS-1, also known as MAVS, Cardif and VISA). RIG-I discerns viral RNA by detecting the 5′-triphosphates of ssRNA and its short double-stranded form, while MDA5 recognizes long double-stranded RNA (Takeuchi and Akira 2008; Saito and Gale 2008). However, the exact element in viral RNA identified by RIG-I and MDA5 is currently unknown. It has been shown so far that genomic RNA of influenza viruses, paramyxoviruses, and HCV and short ($\approx$1 kb) dsRNAs are detected by RIG-I, while picornaviruses such as Encephalomyocarditis Virus (EMCV) and longer ($>$ 2 kb) dsRNA such as poly I:C are detected by MDA5 (Kato et al. 2008; Saito et al. 2008). Both RIG-I and MDA5 signal through IPS-1, which lies on the outer membrane of mitochondria and is associated with Stimulator of IFN Genes (STING; also known as MITA) (Ishikawa and Barber 2008; Zhong et al. 2008) and Fas-Associated Death Domain (FADD) (Balachandran et al. 2004). This complex activates NF-κB and IRF3 via IκK complex and TBK1/IκKi, respectively, initiating pathways that culminate in proinflammatory cytokines and IFN-β production (Fig. 12.1). LGP2 is also an RLR and shares homology with RIG-I and MDA5 in the helicase domain but lacks a CARD domain. It was shown in vitro to be a negative regulator of RIG-I and MDA5; however, results derived from knockout mice suggest that it is actually a positive regulator (Venkataraman et al. 2007; Satoh et al. 2010).

Cytoplasmic DNA recognition is quite distinct from RLR-mediated cytoplasmic RNA recognition. As initially shown by Isaacs et al. (1963) and rediscovered by Suzuki et al. (1999), DNA, especially double-stranded DNA, has been shown to be immunomodulatory. Ishii et al. refined their findings that transfection by natural DNA, or by synthetic polynucleotides that form double-stranded structures,

stimulates cells to produce type-I IFNs and induces cell-autonomous protection from viral replication, independently of TLR9. Unlike the CpG motifs needed for TLR9 activation, methylation of such dsDNA has no effect on activity. Rather, poly (dA-dT) • poly(dT-dA) induces higher levels of type-I IFNs compared with poly (dG-dC) • poly (dC-dG), suggesting that the right-handed helical structure of B-form DNA (B-DNA) is essential for cellular activation of type-I IFNs production; this process is mediated through a TLR-independent, TBK1-dependent means (Ishii et al. 2006). Therefore, TLR-independent, TBK1-dependent cytoplasmic DNA recognition plays an important role in immune responses during viral and bacterial infections (Yasuda et al. 2005; Ishii et al. 2006; Stetson and Medzhitov 2006; Cortez-Gonzalez et al 2006; Martin and Elkon 2006), and in controlling the ensuing adaptive immune responses (Ishii et al. 2008a; Baccala et al. 2007; Babiuk et al. 2004; Spies et al. 2003).

DNA derived from dying host cells can reportedly accumulate when nuclease functions are obstructed, including DNase-I, II, and -III (also known as TREX) (Okabe et al. 2005; Yoshida et al. 2005; Yasutomo et al. 2001; Morita et al. 2004; Napirei et al. 2000). The resultant activation of immune responses through TLR-independent DNA recognition by as-yet undefined receptors can lead to immunological disorders, such as autoimmune diseases (Kawane et al. 2006; Stetson et al. 2008).

Many receptors have been proposed for this TLR-independent, TBK1-dependent type-I IFN production by ds B-form DNA. The first candidate DNA sensor was reported to be DAI (DNA-dependent activator of IFN-regulatory factors), previously called DLM-1 and Z-DNA binding protein 1 (ZBP1) (Takaoka et al. 2007). However, mice lacking DAI (ZBP-1) did not show any expected phenotypes, in vitro or in vivo, suggesting that DAI is not essential for DNA-induced, TBK1-dependent type-I IFN production, or for DNA vaccine immunogenicity (Ishii et al. 2008a). More recent reports suggest that RNA polymerase-III can recognize AT-rich dsDNA, to generate 5′-triphosphate RNA, activating RIG-I in human cells (Ablasser et al. 2009), or in transformed cells (Chiu et al. 2009). Moreover, RIG-I (Choi et al. 2009), HMGB proteins (Yanai et al. 2009), and histone H2B (Kobiyama et al. 2010) were shown to recognize ds B-form DNA and respond by promoting TBK1-dependent type-I IFN production. Although it may take time to resolve how these distinct proteins recognize ds B-form DNA, it will be an exciting field of research.

Recent reports indicate that both type-I IFN (through RLRs) and IL-1β/18 (through inflammasome activation) are engaged in immune response to cytoplasmic nucleic acids (Muruve et al. 2008; Kanneganti et al. 2006a, b). Initially, microbial recognition by innate immune receptors like TLRs induces pro-IL-1β/18 accumulation in the cytosol through MAPK or NF-κB activation. Pro-IL-1β/18 is then cleaved with caspase-1, a major component of all types of inflammasomes, and released as mature form, IL-1β/18. Currently, four types of inflammasome complexes, NLRP1, NLRC4, NLRP3, and AIM2 inflammasome, have been partially characterized. Among them, only NLRP3 and AIM2 appear to be involved in nucleic acid sensing (Vilaysane and Muruve 2009; Franchi et al. 2009).

The NLRP3 inflammasome consists of NLRP3 (also known as NALP3 or cryopyrin), which is an NLR – an Apoptosis-Associated Speck-Like Protein Containing a CARD (ASC) and caspase-1. NLRs are a large family of cytoplasmic sensors, containing 23 members in human and 34 members in mice; their ligands have been only partially elucidated. NLRP3 consists of three domains: C-terminal LRR, central Nucleotide-Binding Oligomerization (NOD) domain and N-terminal ligand-sensing domain, and Pyrin Domain (PYD). A variety of ligands, including nucleic acids and their metabolites, such as RNA, RNA analogs, uric acids crystals, and ATP, are known to trigger NLRP3 inflammasomes (Martinon et al. 2006; Mariathasan et al. 2006; Kanneganti et al. 2006a), inducing formation of inflammasome complexes. These complexes include NLRP3 multimers, the adaptor molecule ASC, and pro-caspase-1 recruited via the ASC CARD domain, leading to autocleavage of caspase-1 (Fig. 12.1). However, it is unclear how NLRP3 detects its nucleic acid ligands.

In contrast to the NLRP3 inflammasome, which is mainly engaged in the recognition of RNAs, such as bacterial and viral RNA, synthetic dsRNA (poly I:C), and ssRNA (imidazoquinoline), the AIM2 inflammasome is activated by ds B-form DNA derived from bacteria, viruses, and host. AIM2 was recently identified as a member of HIN200 protein family; it consists of two domains: HIN200 domain, which binds to cytoplasmic dsDNA , and PYD, which recruits ASC (Roberts et al. 2009; Burckstummer et al. 2009; Fernandes-Alnemri et al. 2009; Hornung et al. 2009). The AIM2 inflammasome leads to activation of caspase-1 in the same manner as the NLRP3 inflammasome (Fig. 12.1).

12.3.2 The Mechanism to Avoid the False Recognition of the Endogenous Nucleic Acids: The Case of Tissue Damage

The innate immune system protects the host against invading infectious agents; however, the same system can also respond to endogenous stimuli (Gallucci et al. 1999; Ishii et al. 2001; Tsan and Gao 2004; Kono and Rock 2008). These stimuli include molecules released from damaged or dying host cells; some are endogenous cytokines and chemokines, including HMGB proteins, IL-1α, and IL-33; the others are heat-shock proteins, hyaluronan degradation fragments, oxidized lipids, nucleic acids, etc. RNA and DNA are normally sequestered tightly in the cells but can be released from host cells in the case of tissue damage, such as necrosis and apoptosis (Matzinger 2002; Ishii and Akira 2005). Nevertheless, nucleic acids are barely recognized by host immune system as there are safety mechanisms. One of these is the limited accessibility to the endosomal compartments where nucleic acid-sensing TLRs are expressed. Another is the immediate elimination of nucleic acids by RNases or DNases within the phagosome, extracellular matrix, or in the serum. A third is the sequential or molecular modification of nucleic acids (Kariko et al 2005). The presence of so many safety mechanisms suggests that any nucleic acid

species can be immunostimulatory if not in the right place in the cells or tissues. In fact, endogenous RNA and DNA are quite immunostimulatory if these safety mechanisms are broken – for example, if immune complexes of RNA or DNA with anti-RNA/DNA antibodies or RNPs are exogenously introduced into cells by transfection reagents, or if nucleotides are subjected to nuclease-resistant modification , or host nucleotides are removed or naturally modified. Otherwise, host RNA and DNA are normally inert to our immune system.

12.3.3 The Mechanism to Recognize Endogenous Nucleic Acids and Their Metabolites as Danger Signal: Another Case of Tissue Damage

Normally, extracellular concentrations of nucleic acids are very low. However, several kinds of molecules, including nucleic acids and their metabolites, which are mainly stored inside the host cell, are released from damaged or dying host cells in the case of tissue injury. Aberrant high concentration of extracellular nucleotides, such as ATP and its metabolic end products, uric acids, can be detected by innate immune sensors as danger signals (Mariathasan et al. 2006; Martinon et al. 2006) (Fig. 12.2). These responses culminate in IL-1β/18 production via NLRP3 inflammasome activation. Although several kinds of mechanisms that activate inflammasomes appear to be involved, the ATP-gated P2X7 receptor contained in the P2 receptor, a class of ubiquitous plasma membrane receptors, plays an important role in the upstream signals that trigger inflammasome activation (Kahlenberg et al. 2005; Ferrari et al. 2006; Di Virgilio 2007). Extracellular high concentration of ATP, a predominantly intracellular molecule, stimulates the P2X7 receptor and induces the activation of a cation channel that mediates potassium efflux. It also induces high concentrations of end-product uric acids and generates uric acids crystals, both of which can activate NLRP3 inflammasomes in gout (Martinon et al. 2006). Extracellular ATP is also engaged in the pathogenesis of bronchial asthma (Idzko et al. 2007). Uric acids, ATP, and adenosine – which is degraded from ATP through a cascade of ectonucleotidases, including nucleoside triphosphate diphosphorylase (NTPDase, also called CD39) and 5′-ectonucleotidase (Ecto50NTase, also called CD73) – induce immune responses (Hasko and Cronstein 2004). Extracellular adenosine binds to and activates four G protein-coupled cell surface receptors, A1, A2A, A2B, and A3, contained in P1 receptors, which signal through alterations in intracellular cyclic AMP and Ca^{2+} concentrations.

Nucleotides and their metabolites can both activate immune responses as danger signals and control or suppress immune reactions (Di et al. 2009). Adenosine's interaction with A2A receptors, which are the predominant subtype in immune cells, may inhibit inflammation by cAMP induction. For instance, hypoxia-inducible factor-1α (HIF-1α) mediates 5′-nucleotidase induction following accumulation of extracellular adenosine; therefore, hypoxic conditions can also

induce immunosuppressive effects via the A2A receptor (Ohta and Sitkovsky 2009). Thus, the recovery process from tissue damage is built upon a strict immunomodulatory mechanism.

12.3.4 Endogenous Nucleic Acids Recognition: The Case of Autoimmune Diseases

Despite the mechanisms to prevent false recognition of "self" nucleic acids and their metabolites described above, innate immune recognition of endogenous nucleic acids can induce detrimental adaptive immune response to "self" antigen – the so-called autoimmune diseases (Marshak-Rothstein and Rifkin 2007). Two major mechanisms of "self" nucleic acids have been identified to trigger autoimmune diseases: delayed clearance of damaged host cells, and unfavorable reactions to immune complexes (Fig. 12.3).

Damaged host cells generated from microbial infections and tissue injuries are immediately eliminated by phagocytes in normal conditions; however, necrotic cells always lose membrane integrity and unavoidably release intracellular contents,

Endosomal TLRs' recognition of autoantigens including nucleic acids

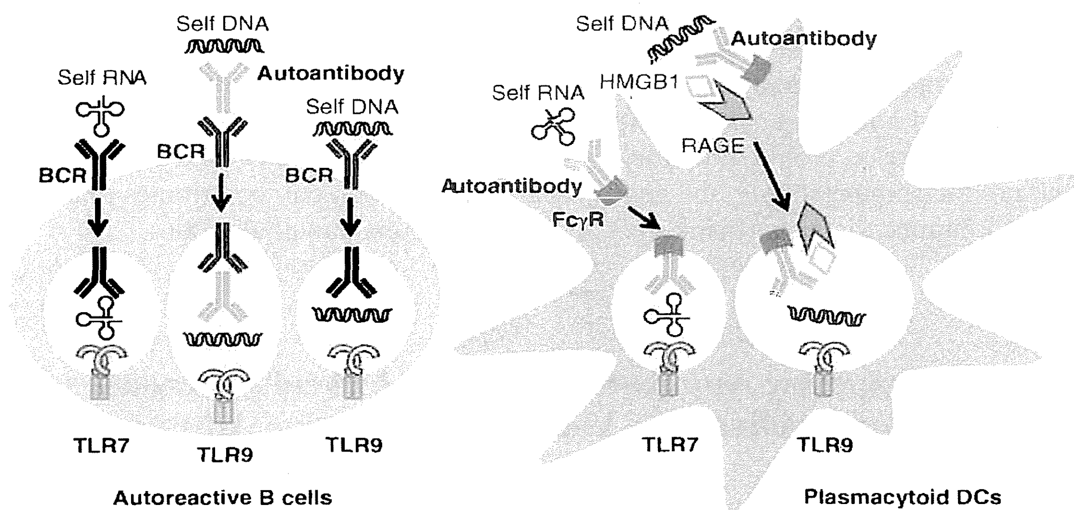

Fig. 12.3 B-cell receptors (BCRs) expressed on the surface of autoreactive B cells can bind autoantigen (DNA or RNA) directly or immune complexes including DNA or RNA and transport both BCRs and autoantigen or immune complexes to the cytoplasmic endosomal compartment containing TLR7/9. pDCs express receptors for the Fc portion of IgG (FcγRs). Immune complexes including DNA or RNA bind FcγRs on pDCs and are transported to cytoplasm. The interaction between HMGB1 (High-Mobility Group Box 1) and RAGE (receptor for advanced glycation end products) also contributes to the recognition of self-DNA-containing immune complexes

inducing immune responses via danger signals (Matzinger 2002). In contrast to necrotic cells, apoptotic cells are strictly cleared through interactions between opsonins and their receptors, such as scavenger receptors, complement receptors on phagocyte cell surfaces (Savill et al. 2002; Erwig and Henson 2008), and the recently identified peroxisome proliferator-activated receptor-δ (PPAR-δ) sensing system (Mukundan et al. 2009). Recent studies also suggest that impaired clearance of nucleic acids in apoptotic cells plays an important role in autoimmunity pathogenesis (Kawane et al. 2006; Napirei et al. 2000; Stetson et al. 2008).

Autoreactive B cells can bind autoantigens released from dying host cells via specific B-Cell Receptors (BCR), which are shown to exist in 5–20% of healthy individuals (Wardemann et al. 2003), and in a greater percentage of autoimmune-disease patients, due to defective early B-cell tolerance (Yurasov et al. 2005). Once these autoantigens are endocytosed by BCR, nucleic acids contained in the cells can stimulate endosomal TLR-7 and -9. These immune system activations, especially type-I IFNs responses via TLRs, play a key role in autoantibody production from proliferated and differentiated autoreactive B cells (Le Bon and Tough 2002; Theofilopoulos et al. 2005; Marshak-Rothstein and Rifkin 2007). B cells also express TLR-9 and the antigen receptor for self-immunoglobulin-gamma (IgG). Both are engaged in the recognition of IgG2a-chromatin immune complexes and induce production of a class of autoantibodies known as Rheumatoid Factors (RF) (Leadbetter et al. 2002). Both B cells and pDCs are involved in recognition of immune complexes – including self-RNA and DNA. PDCs express FcγRs (also known as CD32) to detect the Fc portions of autoantibodies, and Receptor for Advanced Glycation End Products (RAGE), to detect extracellular High-Mobility Group Box-1 (HMGB1) derived from necrotic cells (Tian et al. 2007). These interactions induce engulfment of immune complexes and stimulate TLR-7/8 and -9 in endosomal components, as with B cells.

Microbial infections can also trigger or accelerate a detrimental cascade mediated through increased proinflammatory cytokines (Munz et al. 2009) and damaged host cell products; interestingly, the gene dosage of TLR-7 directly contributes to the risk of autoimmune diseases (Pisitkun et al. 2006; Subramanian et al. 2006).

12.4 Therapeutic Applications of Nucleic Acids as Innate Immune Activators: Vaccine and Vaccine Adjuvants (Table 12.2)

As described above, extracellular nucleic acids and their metabolites can induce both immune activation and suppression via immune receptors, suggesting that nucleic acids and their analogs are candidates for therapeutic agents against infectious diseases, such as vaccines and vaccine adjuvants (Table 12.2) as well as therapies for autoimmune diseases and allergies. Here, we discuss vaccines and vaccine adjuvants that utilize the immunostimulatory effect of nucleic acids: DNA

Table 12.2 Nucleic acids based vaccines and vaccine adjuvants

Vaccine/Vaccine adjuvants (Formation of nucleic acids)	Receptors and adaptors involved in the recognition
Inactivated influenza whole virus vaccine (ssRNA)	TLR7/8-MyD88, RIG-I-IPS-1, NLRP3-ASC
Imidazoquinolines (ssRNA)	TLR7/8-MyD88, NLRP3-ASC
Poly I:C, Poly I:C[12]U (dsRNA)	TLR3-TRIF, MDA5-IPS-1, NLRP3-ASC
CpG oligonucleotides (ssDNA)	TLR9-MyD88
Poly dA:dT (dsDNA)	Unknown-STING-TBK1
DNA vaccine (dsDNA)	Unknown-STING-TBK1

Underlined innate immune signalings are essential for their immunogenicity
Poly I:C: polyinosinic:polycytidylic acid, STING: stimulator of interferon genes

vaccine (dsDNA), inactivated Whole Virus Influenza vaccine (influenza WV) (ssRNA) and poly I:C (dsRNA). CpG-ODNs (ssDNA), which are also very good candidates for therapeutic agents, are discussed in detail in the next section (Klinman 2004).

DNA vaccines are DNA plasmids encoding target antigen genes. Once these vaccines are administered, target antigens are expressed in host cells, inducing adaptive humoral and cellular immune responses. The immunogenicity of DNA vaccine was recently shown to depend on plasmid dsDNA (but not unmethylated CpG-motifs) and mediated through the DAI-independent, TBK1-dependent signaling pathway (the sensor for DNA vaccine has yet to be identified) (Ishii et al. 2008a). On the other hand, the immunogenicity including B cells and Th1-type CD4T cells activation of the influenza WV is dominantly controlled by a TLR7/MyD88-dependent signaling pathway, although TLR7/MyD88, RIG-I/IPS-1, and inflammasome activation are shown to be involved in live influenza virus infection (Allen et al. 2009; Koyama et al. 2007; Thomas et al. 2009; Ichinohe et al. 2009; Koyama et al. 2009). It suggested that genomic ssRNA remaining in influenza WV is essential for vaccine efficacy as an immune activator, detectable by only TLR but not RLR or NLR (Koyama et al. 2010).

A vaccine adjuvant is a compound that promotes and modulates vaccine immunogenicity. In theory, all PAMPs and DAMPs could be candidates for vaccine adjuvants (if safety were not an issue). Poly I:C, a synthetic dsRNA, is a vaccine adjuvant commonly used in animal models. Poly I:C can be detected by both endosomal TLR3 and cytoplasmic MDA5 (Miyake et al. 2009; McCartney et al. 2009); its adjuvanticity is mediated via both pathways (Kumar et al. 2008). The adjuvanticity of poly I:C (Longhi et al. 2009), DNA vaccines (Ishii et al. 2008a), and influenza WV (Koyama et al. 2010) requires dendritic cell activation and type-I IFNs production.

These studies demonstrate that specific signaling pathways in specific host cells play key roles in the efficacy of nucleic acids-based vaccines and vaccine adjuvants, though they can stimulate multiple immune sensors. Therefore, improved efficacy of vaccines and clinical applications require that target antigens and adjuvants be delivered to key immune cells, utilizing various manipulations of drug delivery systems, vaccine formation, routes of administration, and so on.

12.5 Conclusions

Nucleic acids and their metabolites are recognized by the specific host receptors such as TLRs, RIG-like receptors (RLRs), and NLRs, purinergic receptors such as P2X and P2Y receptors, and adenosine receptors such as A2A receptors. Resultant responses vary and may contribute to host defenses, aid homeostatic clearance of dying host cells, or even promote deleterious autoimmune diseases. As more questions emerge about nucleic acids and their relationships with the immune system, more efforts will be needed to elucidate the mechanism of immune recognition of, and regulation by, poly- and oligonucleotides such as RNA and DNA and their metabolites – including mononucleotides, nucleosides, bases, sugars, and uric acids – and the therapeutic potential of nucleic acids and their metabolites.

Acknowledgements The authors thank all members of Laboratory of Host Defense and Department of Molecular Protozoology in the Research Institute for Microbial Diseases, Osaka University. This work is supported by JSPS Research Fellowships for Young Scientists (S.K.), the Knowledge Cluster Initiative (K.J.I.), Grant-in-Aid for Scientific Research (KAKENHI) (K.J.I.), Target protein project (K.J.I. and S.A.), the Ministry of Education, Culture, Sports, Science and Technology in Japan and by CREST, JST (K.J.I.).

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粘膜アジュバント

はじめに

粘膜は生体内において，外来生物，食べ物，代謝産物などの物理的・化学的ストレスに常にさらされながら第一線で防御の役割を担っている．さらにあるときは病原微生物の侵入を認識し，それらを排除する免疫を誘導する一方，常在菌に対して不必要な免疫応答は誘導しない，というように巧妙な仕組みによって免疫応答を使い分けている．そしてこのような繊細な仕組みの破綻が，アレルギー・自己免疫疾患などの疾患発症とも関連していることはすでに10章で概説した．このような意味で，ワクチンによって粘膜免疫をコントロールすることは，単に感染症の予防や治療にとどまらず，アレルギー・自己免疫疾患の病態解明や治療にも関連している．

アジュバントとは，ラテン語の“促進する”“増強する”という意味をもつ“adjuvare”に由来し，もともとは標的抗原とともに投与して，その抗原に対する免疫原性を増強する目的で使用された．

アジュバントに関する報告は，19世紀末にまでさかのぼるが，1920年代にRamonやGlennyらがaluminum hydroxide（alum）を用いてジフテリアや破傷風の類毒素の免疫原性を改善したことによって，アジュバントの重要性が認識されるようになった．これまでアジュバントの作用機序に関しては，非特異的に標的抗原を投与部分に長期間とどめる作用や抗原提示細胞の遊走を促進する作用などによって抗原提示の確率を高くして免疫原性を増強することが想定されていた．しかしながら，近年の自然免疫学の進歩に伴い，アジュバントの多くがToll-like receptors（TLRs），retinoic acid-inducible gene（RIG）-like receptors（RLRs），nucleotide-binding oligomerization domain protein（NOD）-like receptors（NLRs）などの自然免疫受容体に特異的に作用して樹状細胞（dendritic cell：DC）を中心とした抗原提示細胞を活性化し，その遊走や成熟，抗原提示能や補助シグナル分子の発現を促進し，T細胞やB細胞の抗原特異的な活性化を増強することが明らかになった（図1）[1,2]（4章a参照）．そしてアジュバントによる作用は単に免疫原性の増強によって，標的抗原の必要量を減少させたり，接種の回数を減少させたり，免疫力の弱い新生児や高齢者への効果を改善したりするだけにとどまらず，その種類や組み合わせによっては，主に抗体産生（B細胞活性）を誘導するもの，Th1型を誘導するもの，Th2型を誘導するもの，または細胞傷害性T細胞（cytotoxic T lymphocyte：CTL）の活性を誘導するものといったように獲得免疫の方向性をも制御することができる．つまり，ワクチンによって粘膜免疫をコントロールするためにはアジュバントの理解は必要不可欠のものと考えられる．

アジュバントの作用機序をよく理解し，メカニズムに基づいた利用が可能になれば，病原微生物や疾患の特性に応じて，たとえばウイルス感染に対する抗体誘導，悪性腫瘍に対する細胞傷害性T細胞の誘導，アレルギー疾患に対するTh2の抑制などのように，疾患に応じたアジュバントの組み合わせを選択することが可能になり，有効な免疫療法のツールになることが考えられる．