Importance of Nucleic Acid Recognition in Inflammation and Autoimmunity

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Abstract

An important concept in immunology is the classification of immune responses as either innate or adaptive, based on whether the antigen receptors are encoded in the germline or generated somatically by gene rearrangement. The innate immune system is an ancient mode of immunity, and by being a first layer in our defense against infectious agents, it is essential for our ability to develop rapid and sustained responses to pathogens. We discuss the importance of nucleic acid recognition by the innate immune system to mounting an appropriate immune response to pathogens and also how inflammation driven by uncontrolled recognition of self-nucleic acids can lead to autoimmune diseases. We also summarize current efforts to either harness the immune system using agonists of nucleic acid–specific innate sensors or, on the contrary, by using inhibitors in autoimmune situations.

INTRODUCTION

The innate immune system recognizes a broad range of molecular structures that are conserved products present in various microorganisms, and are often referred to as pathogen-associated molecular patterns. This recognition depends on a diverse set of germline-encoded receptors, termed pattern recognition receptors (PRRs) (1). These PRRs include a variety of molecules, such as mannose receptors, glucan receptors, scavenger receptors, and Toll-like receptors (TLRs), as well as intracellular sensors, such as the retinoic acid inducible (RIG)-I-like receptors (RLRs), and DNA sensors. PRRs recognize diverse structures, such as mannose, lipopolysaccharide, lipoteichoic peptidoglycans, flagellins, lipoproteins, and nucleic acids. Recognition of these microbial molecules can then trigger a rapid response, leading to a burst of proinflammatory cytokines and type-I interferons (IFNs), as well as stimulation of professional antigen-presenting cells that initiate and instruct the subsequent adaptive immune response. Nucleic acids have an especially important role as ligands for PRRs because the immune system is primed to respond to infection by microbial DNA and RNA. Sensors of nucleic acids are present in the endosomes of cells and also in their cytosol, and the cellular distribution and redundancy of these sensors allow the maximum protection against pathogens.

MECHANISM OF NUCLEIC ACID RECOGNITION BY THE IMMUNE SYSTEM

Importance of Nucleic Acid Recognition

Nucleic acid recognition by a series of innate receptors leads to a potent activation of the innate immune system and the subsequent production of proinflammatory mediators, such as the type I IFNs. During the past 15 years, following the identification of TLR9 as a receptor for bacterial DNA (2), there has been remarkable progress in our understanding of how cells of the immune system have evolved to recognize pathogens via their genomes or the nucleic acids that are produced during their replication. The importance of nucleic acid recognition is illustrated by a broad redundancy among the many different types of receptors that are expressed by different cells and signals using distinct or, in some cases, redundant signaling pathways. Although pathogens are constantly evolving, nucleic acids are an intrinsic part of their structures, so it is not surprising that the immune system has built a series of tools to sense nucleic acids, and these are an important part of its arsenal for responding to pathogens. The nucleic acid sensors can be divided into two classes based on their location in either the endosomes or the cytosol. The recognition of nucleic acids in endosomes allows for the recognition of viruses that typically uncoat their genomes in endosomal compartments. Once the viruses enter the cytoplasm, they are recognized by cytosolic sensors. Both of these pathways unleash stimulation of a powerful set of inflammatory cytokines, including type I IFNs, as well as inflammatory cytokines such as tumor necrosis factor (TNF), interleukin (IL)-6, IL-1, and IL-18.

The Endosomal Sensors of Nucleic Acids

The past decade has seen a rebirth of interest in innate immunity (3, 4), catalyzed in large part by studies of PRRs (1, 5). PRRs can be divided into five major groups: NOD-like receptors, C-type lectin-like receptors, RIG-I-like receptors, cytosolic DNA receptors, and TLRs. The discovery of TLRs as important components of pathogen recognition has brought new understanding of the key signaling molecules involved in innate immune activation. TLRs are type 1 glycoproteins and are among the most widely expressed recognition receptors of the innate immune system (1, 5).

Following the discovery of the critical antifungal *toll* gene in *Drosophila* (6, 7), the use of database searches has led to the identification of 10 homologous TLRs in humans (8–10). Strikingly, 4 of the 10 identified TLRs in humans recognize nucleic acids (1, 11, 12), and all are present in the endosomes of cells, likely to prevent unwanted recognition of extracellular self-nucleic acids (13). TLR3 recognizes double-stranded RNA (dsRNA) and plays a prominent part in mice during infection with multiple microbes. In humans, TLR3 is widely expressed in blood cells, although it is not present in plasmacytoid dendritic cells (PDCs) and neutrophils (14, 15). Surprisingly, patients deficient for TLR3 have a relatively selective immune deficiency, being susceptible to herpes simplex encephalitis (16). TLR7 and TLR8 recognize single-stranded RNA (ssRNA) but, although they share ligands and have related sequences, their biology is quite different. This can be explained by differences in the distribution of their expression in specific cell types (17–20). TLR7 is restricted to B cells and PDCs in humans, with little expression in conventional dendritic cells and macrophages, and TLR7 induces a dominantly type I IFN-associated response. TLR8 is highly expressed in myeloid cells, including neutrophils, but is absent from PDCs. Similar to TLR7, TLR9 is highly expressed in PDCs and B cells, and induces large amounts of type I IFN (21). TLR9 recognizes bacterial and viral DNA and synthetic oligodeoxynucleotides (ODNs) containing unmethylated CG dinucleotides. These nucleic acid–specific TLRs have evolved under strong selective pressure, as little-to-no polymorphisms are found in humans (22), suggesting their critical role in protecting the host from pathogens.

The Cytosolic Sensors of Nucleic Acids

As many viruses and their products reach the cytosol, there is a need for PRRs other than TLRs. In contrast to the structurally related TLR family, in the cytosol there are many different types of sensors of nucleic acids. A well-characterized family is the RLRs, which sense atypical RNA from viruses in the cytoplasm of infected cells. The RLR family consists of three members that are DExD/H box RNA helicases, although one of its members (LGP2) lacks the N-terminal CARDs and may act to regulate RIG-I and MDA5 signaling. RIG-I recognizes 5′-triphosphorylated, uncapped ssRNA (23), as well as RNA bearing 5′-diphosphates, which are found in many viruses but not in uninfected cells (24). RIG-I and MDA5 may be involved in the recognition of different RNA viruses because MDA5 does not recognize the 5′-end of the viral RNA, but instead recognizes long dsRNA and branched high-molecular RNA structures. These receptors are widely expressed across tissue and cell types. The recognition of DNA involves sensors with different structures. DNA-dependent activator of IFN-regulatory factors (DAI) was the first of these to be identified, and it binds the Z and B forms of DNA. At least 13 distinct proteins have since been proposed to function as cytosolic DNA sensors. These include (in order of their discovery) AIM2 (absent in melanoma-2), RNA polymerase III, LRRFIP1, DHX9, DHX36, IFI16, Ku70, DDX41, DNA-PK, MRE11, Rad50, and cyclic GMP–AMP (cGAMP) synthase (cGAS) (reviewed recently in 25). IFI16 and AIM2 are both members of the PyHIN (pyrin and HIN200 domain–containing) protein family, and bind double-stranded DNA (dsDNA) via HIN200 domains. Although IFI16 will trigger the production of IFN, AIM2 promotes the assembly of an inflammasome complex, leading to caspase-1 activation and subsequent secretion of IL-1β and IL-18. Following DNA-induced dimerization, cGAS produces the cyclic dinucleotide, cGAMP. In turn, cGAMP activates the endoplasmic reticulum–tethered adaptor protein, stimulator of interferon genes (STING), resulting in transcription of IFN-β. Other molecules, in particular helicases such as DDX9 and DDX36, have been associated with DNA recognition in PDCs (26). The detection of multiple sensors of nucleic acids in the cytosol could indicate cell type–specific roles for these molecules. Much remains to be learned in this very active field of research.

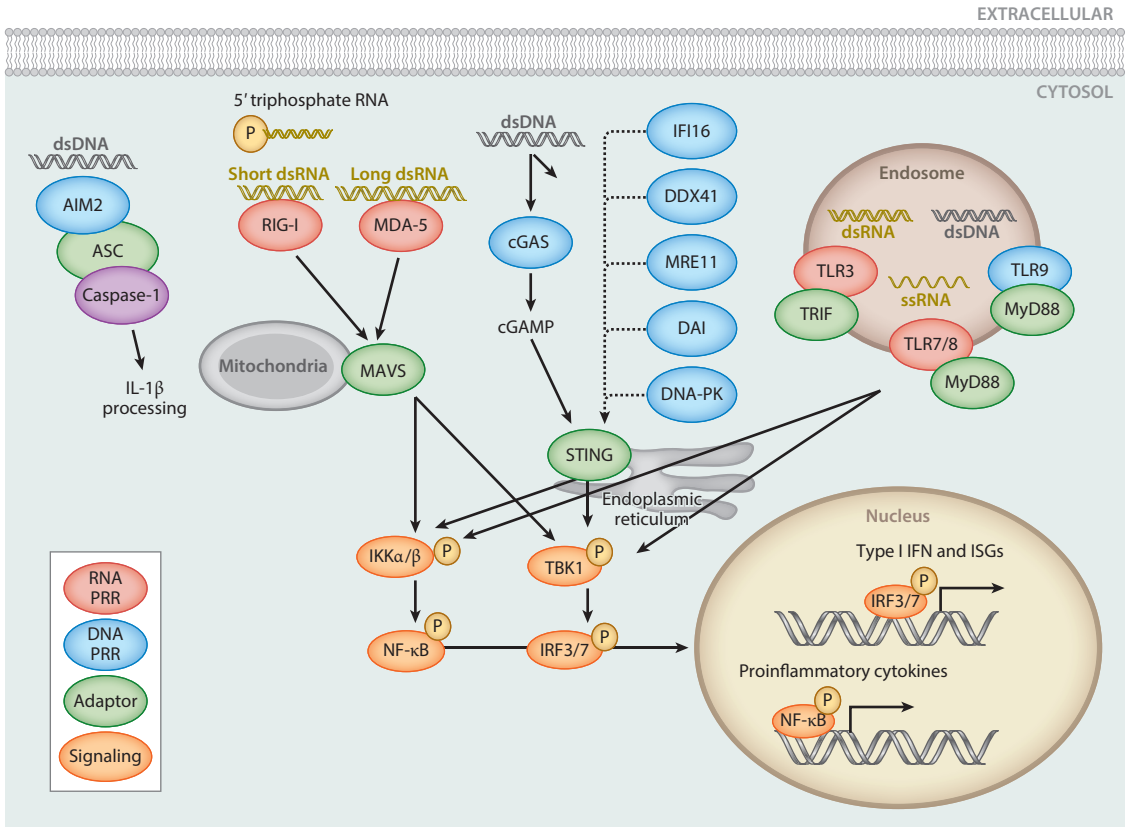

Figure 1

Sensors of nucleic acids and the main components of their signaling pathways. Sensors are present not only in endosomes but also in the cytoplasm of the cells and engage different but overlapping signaling pathways. Abbreviations: AIM2, absent in melanoma-2; cGAMP, cyclic GMP–AMP; cGAS, cGAMP synthase; DAI, DNA-dependent activator of IFN-regulatory factors; ds, double-stranded; IFN, interferon; IL, interleukin; IRF, IFN regulatory factor; ISG, IFN-stimulated gene; MyD88, myeloid-differentiation primary response protein-88; PRR, pattern recognition receptor; RIG-I, retinoic acid–inducible gene I; ss, single-stranded; STING, stimulator of IFN genes; TLR, Toll-like receptor.

Nucleic Acid–Induced Signaling Pathways and Cellular Expression

The response to nucleic acids is regulated not only by the signaling pathways induced by both cytosolic and endosomal receptors but also by their cellular expression and regulation. With the exception of TLR3, all TLRs use the widely associated adaptor protein myeloid-differentiation primary-response protein-88 (MyD88) (**Figure 1**). MyD88 then activates IL-1R-associated kinase (IRAK)-1 or IRAK-2, or both, and also IRAK-4. This leads to the activation of TRAF6 and the involvement of the NF-κB pathway. In addition, these TLRs can alternatively engage a different pathway than NF-κB that leads to high levels of type-I IFN, through either the activation and nuclear translocation of IFN regulatory factor (IRF)-3 in the cases of TLR3 and TLR4 (27) or IRF7 in the cases of TLR7, 8, and 9 (28). Other molecules are involved as well, such as the δ subunit of the PI3 kinase, which is an important component of the TLR7 and TLR9 pathway leading to IFN-α production in human PDCs (29), or Btk, which is key for TLR9 but not TLR7 signaling in PDCs (30). The adaptor molecules used by a TLR are not the sole determinant of

the signal transduction pathways stimulated by a particular TLR. For example, in both mouse and human PDCs, the regulation of IRF7 versus NF-κB pathways following TLR9 activation is regulated by recognition of the TLR in distinct endosomal compartments (31, 32). TLR3 uses a different adaptor, named TIR domain–containing adaptor protein–inducing IFN-β (TRIF) (5). TRIF then activates TANK-binding kinase-1 (TBK1) and IKKi (also called IKKε), which phosphorylate IRF3 and IRF7. The cytosolic RLRs interact with IPS1 (also called MAVS), which activates the IKK-related kinase, TBK1 and IKKi, and subsequently the transcription factors IRF3 and IRF7. As mentioned above, a key molecule involved in the sensing or response to DNA is STING, which triggers the activation of TBK1 and IRF3, as well as the canonical NF-κB pathway. Interestingly, the engagement of MAVS, STING, or TRIF molecules leads to the activation of TBK1/IRF3, which will induce IFN. Furthermore, patients who are deficient for either MyD88 or IRAK-4 cannot produce IFN in response to TLR agonists, but are still able to control virus infections (33), which reiterates the importance of the redundancy that has been built by the innate immune system to control virus infections.

IMPORTANCE OF NUCLEIC ACID RECOGNITION DURING ANTIVIRAL IMMUNE RESPONSE

The host PRRs described above collectively serve as a viral sensor, surveying distinct cellular compartments for signs of viral infection. Although some PRRs detect viral proteins, the primary means of sensing viruses is via detection of their genomes or nucleic acids generated during their replication. TLR3, TLR7/8, and TLR9 sense viral nucleic acids. The role of TLR3 (a sensor of dsRNA) (14, 15) in antiviral immunity is complex and differs greatly depending on the viral challenge. TLR3 is antiviral in the context of multiple viruses, including murine cytomegalovirus (CMV) (34, 35), hepatitis B virus (HBV), Chikungunya virus (36), and encephalomyocarditis virus (EMCV), but it is dispensable for detection of reoviruses, lymphocytic choriomeningitis virus (LCMV), or vesicular stomatitis virus (VSV) (37). In contrast, engagement of TLR3 worsens pathology during infection with West Nile virus (38) or Punta Toro virus (39). TLR3 has pro- and antiviral effects in the context of influenza A virus. As mentioned above, TLR3 has been linked to susceptibility to herpes simplex encephalitis (16), as well as dengue hemorrhagic fever (40).

TLR7 has been shown to drive IFN-α production in PDCs exposed to live and heat-inactivated influenza virus (41), as well as following infection with VSV (42) and Sendai virus (43). TLRs can also sense viral nucleic acids from viruses that replicate in the cytoplasm via an autophagy mechanism that delivers cytosolic nucleic acids to endosomal TLRs (43). TLR9 has been shown to have a crucial role in infections caused by a number of DNA viruses, including the herpesviruses CMV, herpes simplex viruses types 1 and 2, Kaposi's sarcoma-associated herpesvirus, murine herpesvirus 68, and Epstein–Barr virus (34, 44, 45).

Extensive functional studies conducted primarily in mouse models have shown that RIG-I and MDA5 sense different classes of RNA viruses, with RIG-I having a critical role in the detection of orthomyxoviruses, rhabdoviruses, and arenaviruses, and MDA5 preferentially detecting picornaviruses. Many viruses (flaviviruses, paramyxoviruses, and reoviruses) are thought to be sensed by both RIG-I and MDA5. The recognition of dsDNA from DNA viruses leads to robust induction of both type-I IFNs and production of the IL-1 family of proinflammatory cytokines (46). The IL-1 family members IL-1β and IL-18 have a central role in host defense against a variety of viruses (47). IL-1β and IL-18 are synthesized as inactive proteins following the stimulation of PRRs, such as the TLRs (48). AIM2 then recognizes viral dsDNA and triggers the assembly of an inflammasome complex, leading to caspase-1 activation and proteolytic processing of IL-1β and IL-18 (49–52). AIM2 is essential for the early control of murine CMV infection in vivo (53).

The STING–TBK1 axis, downstream of the cGAS and other DNA sensors, controls the type-I IFN response and host resistance against a number of DNA viruses, including herpesviruses, adenoviruses, and vaccinia virus (54). Mice deficient for cGAS are unable to control herpesvirus infection (55), vaccinia virus infection (56, 57), or recognize HIV complementary DNA (56, 58, 59).

DISEASES ASSOCIATED WITH NUCLEIC ACID RECOGNITION

Inflammation from almost any cause leads to the release of cellular debris that includes DNA and RNA. Whereas extracellular nucleases that help degrade DNA are fairly abundant, self-nucleic acids enter innate immune cells through multiple mechanisms. Because the basic genetic code of nucleic acids is identical in eukaryotes and prokaryotes, the task of self–nonself discrimination is particularly challenging for innate immune cells. Although subtle biochemical modifications contained within host or bacterial nucleic acids may alter sensing by human phagocytes (60, 61), in many cases sensors respond equally well to nucleic acids regardless of their origin. When exposure to nucleic acid is excessive (e.g., through a lack of clearance or impaired degradation of cell debris) or regulation of the response to nucleic acid is poorly controlled, a heightened innate immune response predisposes to autoimmune and autoinflammatory disorders.

Psoriasis

Psoriasis is a common autoinflammatory disease. Psoriasis is characterized by scaling and plaques, particularly in extensor areas of the body. The pathogenesis is multifactorial and includes a genetic predisposition that likely involves both keratinocyte biology and immune responses, as well as environmental factors (62). On histopathologic examination of untreated lesions, thickening of the epidermis is associated with a prominent neutrophilic infiltrate and accumulation of PDCs and myeloid dendritic cells. The detection of both neutrophils and PDCs strongly implicates activation of TLRs in this disease. Among the barrage of inflammatory mediators released by activated neutrophils, two components are particularly important for the generation of TLR ligands: (*a*) the antimicrobial peptide LL37 (the C-terminal peptide of cathelicidin, belonging to the human β-defensin family) and (*b*) the nucleic acids DNA and RNA that bind to LL37. These LL37–nucleic acid complexes can enter PDCs and also keratinocytes (63) to stimulate TLR7 or TLR9, a mechanism similar to that suggested in tissue lesions in systemic lupus erythematosus (SLE) (64). Although this pathway of intracellular activation is difficult to prove in humans in vivo, additional support comes from a mouse model of psoriasis that is induced by the TLR7 agonist imiquimod (IMQ). Topical IMQ applied to mouse skin results in scaly lesions associated with epidermal proliferation, and the accumulation of neutrophils, similar to psoriasis (65). IMQ stimulates the local production of cytokines such as type-I IFN, TNF, IL-12, IL-1, and IL-6; recruits inflammatory cells including PDCs; and culminates in the activation of the adaptive immune response, characterized by a dominance of Th1 and, to a lesser extent, Th17 responses (65). Interestingly, after the protracted topical application of IMQ, FVB/N mice—but not mice lacking TLR7—developed a lupus-like disease that was dependent on PDCs (66), indicating that chronic activation of TLR7 could mediate SLE in susceptible genetic backgrounds.

Systemic Lupus Erythematosus and Related Disorders

SLE (or lupus) is a complex autoimmune disease that affects multiple organs and is marked by periods of disease remission and flare (67). Multiple abnormalities contribute to the pathogenesis of SLE. These include defective clearance of immune complexes and dead and dying cells; exaggerated responses to nucleic acid antigens, leading to inflammatory cytokine and chemokine

production; and altered thresholds of activation of B and T lymphocytes. In particular, the presence of elevated levels of type I IFN is a hallmark of the disease, and this pathway is generating great interest, with many novel therapeutics currently in trials (89–92). Cumulatively, these abnormalities lead to a loss of self-tolerance and to the production of autoantibodies. Autoantibodies in SLE are directed against nucleic acids and associated nuclear proteins (chromatin), as well as ribonucleoproteins. Although it is well known that tissue damage is mediated by the deposition of pathogenic autoantibodies and immune complexes in the affected organs, how the innate immune system becomes activated and generates the IFN signature is less clear. The nucleic acid–sensing intracellular TLR7 and TLR9 are expressed at the highest levels in B cells and PDCs, which may help to explain why these cells play such a prominent part in the pathogenesis of SLE. Mice that overexpress TLR7 develop a lupus-like disease, and lupus-prone mice that are rendered deficient in TLR7 are protected from lupus (68). TLR9 has a more complex role: It influences anti-DNA responses, but unexpectedly, TLR9-deficient lupus-prone mice develop worse disease, possibly related to exaggerated TLR7 signaling (68). In humans, two pathways are implicated in the activation of TLR7 and TLR9. The first relates to the LL37 pathway (69) and also to other "schleppers" such as HMGB1, which allow the transfer of nucleic acids into the cell. A second well-defined pathway is the entry of immunoglobulin (Ig)G antibodies bound to DNA or RNA through FcgR2A into PDCs, leading to TLR7- and/or TLR9-induced type-I IFN (64, 70–72), contributing to the typical IFN signature seen in the peripheral blood of most lupus patients (73–77). If and how this signature is generated prior to the presence of IgG immune complexes remain to be determined.

Interferonopathies

Aicardi–Goutières syndrome and spondyloenchondrodysplasia are two examples of rare, monogenic disorders belonging to the syndrome of interferonopathies. These diseases present in infancy or early childhood and are characterized by a variety of clinical manifestations, autoimmunity, and increased production of type-I IFN, as determined by increased expression of IFN-stimulated genes in peripheral blood cells (78). Identification of the genes responsible for the interferonopathies has been highly informative in providing insight into how individual nucleic acids stimulate immune activation and inflammation. For example, Aicardi–Goutières syndrome, a pediatric disease characterized by encephalopathy and skin manifestations, is caused by mutations in at least seven genes that are responsible for processing nucleic acids (78). In contrast to psoriasis and SLE, discussed above, the nucleic acid ligands are thought to arise from intracellular sources and to predominantly trigger non-TLR sensors in the cytosol. Highlighting the significance of the STING pathway, discussed above, a recently described pediatric disease, known as SAVI (STING-associated vasculopathy with onset in infancy), characterized by severe interstitial lung disease and cutaneous vasculopathy, has been shown to be caused by activating mutations of STING (79). Recent analysis of STING-deficient lupus-prone mice has revealed that they develop worse disease, suggesting that in systemic autoimmunity, STING pathways may have more complex roles (80).

THERAPEUTIC POTENTIAL OF TARGETING NUCLEIC ACID SENSORS

Agonists of Nucleic Acid Sensors

Being critical players of the immune system, sensors of nucleic acids have become obvious targets in a number of clinical indications (**Table 1**). The only drug targeting these sensors that has been

Table 1 Clinical development of agonists and antagonists of nucleic acid sensors in human diseases

Targeted receptor	Compound (name)	Company	Indications
TLR3	Poly-ICLC (Hiltonol)	Oncovir	Cancer: malignant brain tumors and astrocytoma; glioblastoma (+ temozolomide); non-small-cell lung carcinoma (combined with MUC1-targeting vaccination)
			Cancer vaccine: glioblastoma (combined with dendritic cell vaccine)
			Anal dysplasia; smallpox (preclinical)
	Poly I:poly C12U (Ampligen)	Hemispherx Biopharma	Infectious diseases: HPV, HIV, hepatitis, and influenza; chronic fatigue syndrome
TLR7	Imiquimod (Aldara)	Meda AB	Cancer: superficial basal cell carcinoma
			Infectious diseases: genital warts; actinic keratosis
	AZD8848	AstraZeneca and Dainippon Sumitomo Pharma	Asthma and allergic rhinitis (hay fever)
	GSK2245035	GlaxoSmithKline	Allergic airways diseases; asthma
	GS-9620	Gilead	Infectious diseases: HBV, HCV, and HIV infections
	TMX-101 (Vesimune)	Telormedix	Cancer: non-muscle invasive bladder cancer
TLR7 and TLR8	R848 (resiquimod)	Meda AB	Cancer vaccine: adjuvant in cancer vaccines for multiple types of tumors
TLR8	VTX-2337 (Motolimod)	VentiRx	Cancer: squamous cell carcinoma of the head and neck (+ cetuximab); ovarian cancer
	VTX-1463	VentiRx	Allergic rhinitis
TLR9	Kappaproct	InDex Pharmaceuticals	Ulcerative colitis
	DIMS 9054	InDex Pharmaceuticals	Pulmonary inflammation; multiple sclerosis (preclinical)
	MGN1703 (dSLIM)	Mologen	Cancer: colorectal cancer; lung carcinoma (small-cell bronchial carcinoma)
	MGN1601	Mologen	Cancer vaccine: MGN1703 plus allogeneic cancer cells; renal cell cancer
	AZD1419	Dynavax and AstraZeneca	Asthma
	SD101/CpG-C	Dynavax	Cancer: low-grade B cell lymphoma (combined with low-dose radiation or + ipilimumab); metastatic melanoma (+ anti-PD-1); relapsed non-Hodgkin's lymphoma
	1018 (Heplisav-B)	Dynavax Technologies	Infectious diseases: TLR9 agonist (1018 ISS) + hepatitis B surface antigen
	CpG 7909 (PF-3512676)	Pfizer	Cancer: mantle cell lymphoma
	CpG 7909 (PF-3512676) + BioThrax	Emergent BioSolutions	Anthrax vaccination
STING	Cyclic dinucleotides (ADU-S100)	Aduro Biotech	Palpable cancer (preclinical)
RIG-I and NOD2	SB 9200	Spring Bank Pharmaceuticals	Infectious diseases: HCV and HBV

(*Continued*)

Table 1 (*Continued*)

Targeted receptor	Compound (name)	Company	Indications
TLR7 and TLR9 antagonist	IMO-3100	Idera Pharmaceuticals	Psoriasis
TLR7, TLR8, and TLR9 antagonist	IMO-8400	Idera Pharmaceuticals	Cancer: Waldenström's; diffuse large B cell lymphoma with the MyD88 (L265P) mutation; psoriasis
TLR3, TLR7, and TLR8 antagonist	RSLV-132	Resolve Therapeutics	Systemic lupus erythematosus
TLR7 and TLR9 antagonist	DV1179	Dynavax	Systemic lupus erythematosus

Abbreviations: HBV, hepatitis B virus; HCV, hepatitis C virus; HPV, human papillomavirus; MyD88, myeloid-differentiation primary-response protein-88; RIG-I, retinoic acid–inducible gene I; STING, stimulator of interferon genes; TLR, Toll-like receptor.

approved by the US Food and Drug Administration remains the TLR7 agonist IMQ (under the name Aldara), but many companies are developing strategies to target one or more of these sensors using synthetic agonists or antagonists (**Table 1**). The targeting of endosomal sensors, such as the TLRs 3, 7, 8, and 9, is the most advanced with respect to clinical development, likely because they were the first to be identified. The primary applications for nucleic acid–sensor agonists are as adjuvants in treating cancer, infectious diseases, and also as immunomodulators in allergy treatment. Agonists for TLR3 are being used in cancer treatment with the objectives that type-I IFN production and the activation of tumor antigen–specific T cells will lead to enhanced tumor killing. The TLR7 agonist IMQ is routinely used for the topical treatment of papillomavirus-induced genital warts, as well as basal cell carcinoma (81). Because of its early approval and the ease of access of the skin where IMQ is applied, many studies have been conducted, and the induction of type-I IFN in the skin favors recruitment to the skin of antigen-specific T cells (82).

CpG-ODNs have been designed based on specific motifs that are known to trigger TLR9 in humans, and they have been constructed with a modified phosphorothioate backbone in contrast to the natural phosphodiester backbone. This modification confers increased activity and, most importantly, stability in vivo. The half-life of these modified ODNs can be multiple days in tissues, compared with hours for natural ODNs, due to their increased resistance to both exonucleases and endonucleases (83). Currently, the most advanced clinical programs are using the adjuvant properties of CpG-ODNs, while other programs use ODNs that induce significant levels of type-I IFN from PDCs for cancer indications. Similar to agonists of TLR7 and TLR8, the ability of TLR9 to induce a Th1 response has also generated interest for the treatment of allergy and asthma. Although the biology of TLR8 is less well understood, due to the lack of a functional receptor in the mouse (20), targeting this other RNA-recognition receptor is also being tested in human trials. Agonists of TLR8 or TLR7/8 (resiquimod) are being developed for cancer treatment and cancer vaccines and also for allergic indications. The rationale is based on the ability of these receptors to skew the response toward a Th1-like response compared with the proallergic Th2 situation. Another major application is to use TLR activation to trigger IFN to prevent viral replication in various infectious diseases (human papillomavirus, HIV, and hepatitis and influenza virus infections).

Synthetic cyclic dinucleotides (CDNs) have also been developed to target STING, with the objective of using for therapeutic applications the formidable adjuvant properties of this pathway and the strong induction of type-I IFN by most cell types. Similarly to the utilization of TLRs,

CDNs have been developed as cancer vaccines, mucosal adjuvants, and also as immunomodulators (84). A recent study has suggested that TLR9 and CDNs together may be synergistic in the induction of Th1 responses and potent antitumor responses (85). Polyinosinic-polycytidylic acid (poly I:C) has been used as an experimental therapeutic to activate the MDA5 pathway. The ligand for RIG-I, 5′ppp-dsRNA, has also been used to enhance vaccination responses in virus-mediated disease (86).

Antagonists of Nucleic Acid Sensors

The innate immune system faces the same fundamental challenge as the adaptive immune system: distinguishing self- from nonself-antigens. The involvement of TLRs in self-recognition is particularly clear for the nucleic acid receptors TLR7, 8, and 9, which appear to mediate the pathogenesis of several autoimmune diseases, such as SLE (13, 87, 88), inflammatory arthritis (20), and various autoimmune inflammatory skin diseases (82). This explains why the first clinical program aimed at blocking nucleic acid sensors has been focused on blocking TLR7 and TLR9 in lupus (DV1179 from Dynavax; **Table 1**). Initial results showed no impact of treatment on the IFN signature in lupus patients; however, it is unclear whether this was due to the nature of the inhibitor or of the target. A similar molecule, IMO-3100 (Idera Pharmaceuticals), showed great promise in psoriasis, pointing to the skin as an interesting target for inhibitors of these receptors. An inhibitor of TLR7, 8, and 9 is currently being tested in patients with diffuse large B cell lymphoma who have mutations in MyD88 that lead to its chronic activation. In addition, there are molecules in preclinical studies that aim to block cytosolic sensors, but these are still in the very early stages of development. Of interest, antimalarial drugs have recently been shown to inhibit DNA-stimulated cGAS–STING stimulations (93). Whether this approach can be harnessed to more effectively treat autoimmune and autoinflammatory diseases remains to be determined.

Degradation of Extracellular Nucleic Acids In Vivo

An alternative to blocking intracellular TLR sensing of nucleic acids is degradation of extracellular nucleic acids prior to cell entry. Because addition of RNase to RNP-containing immune complexes attenuates type 1 IFN production by PDCs in vitro, Sun et al. (94) crossed an RNase transgenic mouse to TLR7 transgenic mice to create a double transgenic (RNase × TLR7 Tg) and showed that double transgenic mice had increased survival and fewer immune deposits in the kidney than TLR7 single transgenic mice. A biologic fusion of RNase and IgG, RSLV132, is currently in phase II clinical trials for SLE (**Table 1**).

CONCLUSIONS

The recognition of nucleic acids by innate immune cells blurs the boundaries of self- and nonself-discrimination because nucleic acids are common both to viruses and to the host cells they infect (95). Nucleic acids from viral genomes or products of viral replication are very potent drivers of immune defenses. This fine balance is easily perturbed, however, resulting in potent immune activation by self-nucleic acids that accrue in endosomal or cytosolic compartments, leading to a myriad of localized and systemic inflammatory diseases. Considerable progress has been made in defining the PRRs that sense nucleic acids, elucidating their signaling pathways, and defining their roles in inflammatory and autoimmune diseases. Detailed understanding of these pathways has unveiled new targets that could be used to intervene in diverse inflammatory diseases.

DISCLOSURE STATEMENT

F.J.B. has been listed as co-inventor on pending and issued patents held by Dynavax Technologies that are related to the use of TLR agonists or antagonists in human diseases. F.J.B. is being paid as a consultant for Biogen Idec and Bullet Biotechnology, and has received research grants from Stemline Therapeutics. K.B.E. holds grants from the National Institutes of Health and the Alliance for Lupus Research. K.B.E. is a cofounder of Resolve Therapeutics. K.A.F. holds grants from the National Institutes of Health.

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