

Intracellular Nucleic Acid Sensors and Autoimmunity

Argyrios N. Theofilopoulos, Dwight H. Kono, Bruce Beutler, and Roberto Baccala

A collection of molecular sensors has been defined by studies in the last decade that can recognize a diverse array of pathogens and initiate protective immune and inflammatory responses. However, if the molecular signatures recognized are shared by both foreign and self-molecules, as is the case of nucleic acids, then the responses initiated by these sensors may have deleterious consequences. Notably, this adverse occurrence may be of primary importance in autoimmune disease pathogenesis. In this case, microbe-induced damage or mis-handled physiologic processes could lead to the generation of microparticles containing self-nucleic acids. These particles may inappropriately gain access to the cytosol or endolysosomes and, hence, engage resident RNA and DNA sensors. Evidence, as reviewed here, strongly indicates that these sensors are primary contributors to autoimmune disease pathogenesis, spearheading efforts toward development of novel therapeutics for these disorders.

Introduction

THE PATHOGENESIS OF AUTOIMMUNE DISEASES has long been addressed from the perspective of abnormalities in the adaptive immune system. Despite impressive advances in defining these abnormalities, a comprehensive picture of how these diseases are initiated has remained relatively difficult to define and integrate into a concise scheme. The recent discovery of an array of cell surface and intracellular germline-encoded innate sensors, which recognize exogenous and endogenous danger signals, has provided a more solid foundation to define the pathogenesis of these disorders. Consequently, it has become clear that, akin to normal adaptive immune responses, pathogenic autoimmune responses almost invariably require a preceding engagement of the innate immune system. Although innate responses are normally beneficial, if excessive or protracted, they can result in pathogenic inflammatory/autoimmune diseases, especially in genetically predisposed individuals. Here, we focus on sensors for nucleic acids, because their ligands are represented in both pathogens and hosts, and many lines of evidence strongly establish them as causative factors for several autoimmune diseases. We describe the diversity of these receptors, their intracellular distribution, trafficking patterns, activation requirements, and the elicited pathogenic mediators.

Complexity of Nucleic Acid Sensors

Cells of the immune system are equipped with a broad range of germline-encoded sensors that recognize primarily microbial substances and, in certain instances, endogenous

products released from damaged cells. This ever-expanding list of sensors includes Toll-like receptors (TLRs), retinoid acid-inducible gene (RIG)-like receptors (RLRs), nucleotide oligomerization domain (NOD)-like receptors (NLRs) (Baccala and others 2009; Barbalat and others 2011; Sharma and Fitzgerald 2011), and several other newly identified receptors. These sensors reside on cell surfaces, the cytosol, or within intracellular organelles, thereby safeguarding the integrity of the host. Although all these sensors may directly or indirectly participate in the pathogenesis of autoimmunity, those recognizing DNA or RNA are more likely to play a critical role, as nucleic acids of foreign origin and self-origin are mostly indistinguishable. In particular, nucleic acid sensors are relevant to the pathogenesis of systemic lupus erythematosus (SLE), in which autoantibodies against nucleosomal and spliceosomal antigens typically predominate.

Endolysosomal Nucleic Acid Sensors

The known innate sensors represented within the endolysosomes are the nucleic acid-sensing TLRs, including TLR3, specific for double-stranded RNA (dsRNA), TLRs 7 and 8 for single-stranded RNA (ssRNA), and TLR9 for DNA (Beutler and others 2006; Uematsu and Akira 2006; Moresco and others 2011) (Fig. 1). These sensors are expressed in several cell types intimately involved in immune responses, including conventional dendritic cells (cDCs), plasmacytoid DCs (pDCs), macrophages, and B cells. The expression profile of nucleic acid-sensing TLRs differs among these cell types, and these differences as well as cell-specific means of transport, intracellular localization, and utilization of TLRs may influence the type of pathogens recognized and the

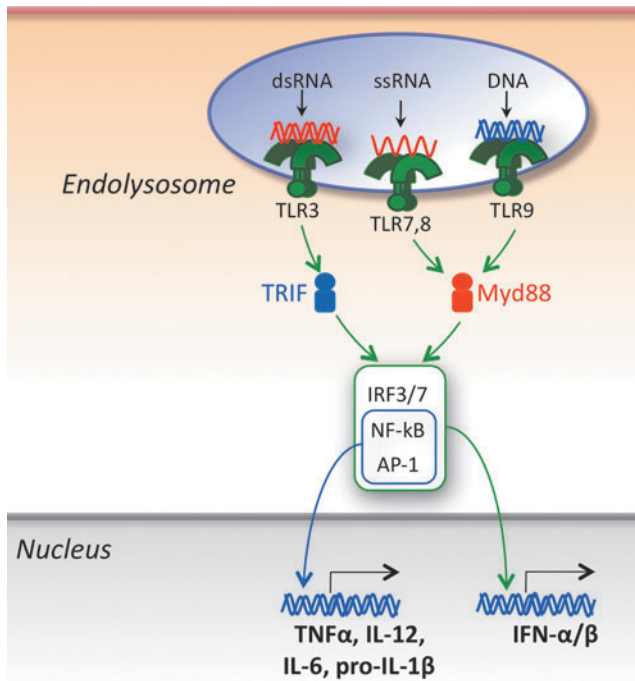


FIG. 1. Endolysosomal nucleic acid sensors. The presence of nucleic acids in endolysosomes is detected by TLR3 (dsRNA), TLR7 (ssRNA), TLR8 (ssRNA), and TLR9 (DNA). Ligand binding induces TLR dimerization and recruitment of the main signaling adaptors TRIF (used by TLR3) and MyD88 (used by TLRs 7, 8, and 9). These adaptors provide a nucleating structure for the formation of higher-order oligomeric complexes composed of kinases, ubiquitin ligases, and other signaling molecules that mediate activation of transcription factors (IRF3, IRF7, NF- κ B, and AP-1) that, on nuclear translocation, promote expression of type I IFNs and other proinflammatory cytokines.

responses elicited. For example, in humans, pDCs selectively express TLRs 7 and 9, whereas cDCs preferentially express TLRs 3 and 8 (in addition to TLRs 1 and 2), monocytes TLR8 (in addition to TLRs 1, 2, 4, and 5), and B cells TLRs 7, 8, and 9 (in addition to TLRs 1, 2, 4, 5, and 6). These distinct TLR expression profiles suggest that efficient innate immune responses require the participation of multiple cell types, rather than a few specialized cells. All TLR family members, including the endolysosomal TLRs, are type I membrane proteins composed of a ligand-binding ectodomain containing several (18 to 25) tandem copies of leucine-rich repeats (LRRs), a single-pass transmembrane domain, and a conserved cytoplasmic Toll/interleukin-1 receptor (TIR) domain for signal transduction. Ligand-induced dimerization and conformational rearrangements of the TIR domains lead to the creation of 2 symmetry-related sites for the binding of the TIR domains of the cognate signaling adaptor molecules (Kawai and Akira 2006; Kenny and O'Neill 2008). Two main adaptors are utilized, ie, myeloid differentiation factor 88 (MyD88) by TLRs 7, 8, and 9, and TRIF (TIR domain-containing adaptor inducing interferon [IFN]- β) by TLR3. These adaptors mediate the recruitment of a series of kinases (IL-1 receptor-associated kinase [IRAK1], TGF β -activated kinase 1 [TAK1], I κ B kinase [IKK] $\alpha\beta\gamma$, mitogen-activated protein kinases [MAPKs], TANK-binding kinase 1 [TBK1], IKK ϵ) and ubiquitin ligases (TNF receptor-associated factor

[TRAF3 and TRAF6]), thus leading to the formation of specific macromolecular signaling platforms. The subunit stoichiometry of one of these platforms, termed Myddosome, created by homotypic interactions of the death domains of MyD88 and the kinases IRAK4 and IRAK2 (or the related IRAK1) has been defined (Motshwene and others 2009; Lin and others 2010; Gay and others 2011; Nagpal and others 2011). The TLR signaling cascade results in the activation and nuclear translocation of several transcription factors, including nuclear factor κ B (NF- κ B), adaptor protein 1 (AP-1), IFN-regulatory factor 3 (IRF3), and IRF7, which together initiate the expression of genes encoding type I IFNs and other cytokines, chemokines, chemokine receptors, and costimulatory molecules. Secreted type I IFNs bind to a specific receptor (IFNAR) present in nearly all cell types and trigger a large number of IFN-stimulated genes through the Janus kinase (JAK)/signal transducer and activator of transcription (STAT) pathway (Schoggins and others 2011).

As is the case for TLR4, which requires the serum lipopolysaccharide (LPS)-binding protein as an accessory molecule for LPS binding, a recent study identified granulin, a cysteine-rich serum factor, as a specific contributor to CpG-containing oligodeoxynucleotides (CpG-ODN)-induced TLR9 signaling and production of proinflammatory cytokines by pDCs and cDCs (Park and others 2011). A model proposed to explain this finding is that CpG-ODN binds to granulin or its precursor, progranulin, and the resulting complex is delivered to endolysosomes on interaction with the lysosomal sorting protein sortilin (Moresco and Beutler 2011).

An unexpected recent finding was that major histocompatibility complex (MHC) class II molecules are required for optimal TLR signaling and induction of type I IFNs and other proinflammatory cytokines (Frei and others 2010). This positive regulation, taking place in endosomes, is mediated by the interaction of MHC class II molecules, via CD40, with the Bruton's tyrosine kinase, which then interacts with the adaptors MyD88 and TRIF (Hassan and Mourad 2011; Liu and others 2011b).

TLR signaling is modulated by multiple endogenous negative regulators that act at the cell membrane or intracellularly by interfering with adaptors, kinases, and transcription factors (Lang and Mansell 2007; Watters and others 2007; Ananieva and others 2008; Lemke and Rothlin 2008; Coll and O'Neill 2010). Inhibitors of TLR signaling may also be encoded by viral and bacterial genes (Stack and others 2005; Cirl and others 2008). Whether alterations in the expression and/or function of these negative regulators play any role in the pathogenesis of autoimmune diseases remains to be fully characterized, but mice deficient in some of these inhibitors, ie, SIGIRR (single Ig interleukin 1 [IL-1] receptor related) or Tyro, Axl and Mer (TAM) receptor tyrosine kinases, have been reported to develop lupus-like manifestations (Rothlin and others 2007; Lech and others 2008).

TLR compartmentalization and trafficking

Spatiotemporal regulation of intracellular trafficking is a determining factor for TLR accessibility to ligands, intensity of signals, and quality of the inflammatory responses, as has been illustrated for the high efficiency of type I IFN production by the TLR7- and TLR9-expressing pDCs (Honda and others 2005). TLRs 3, 7, 8, and 9 are localized in several intracellular compartments, including the endoplasmic

reticulum (ER), the endosomes, and lysosomes. Activation of these TLRs, however, occurs only in acidified endolysosomes, as responses are extinguished when acidification is prevented (Hacker and others 1998). These results pose a question of how cells sense nucleic acids before the ER-resident TLRs are mobilized to the acidified endolysosomal compartment. One potential answer is that minimal trafficking of these TLRs takes place at a steady state or in response to low-grade inflammatory stimuli. Indeed, the type of carbohydrates on intracellular TLRs 7 and 9 in non-stimulated cells suggests that these TLRs have trafficked from the ER through the Golgi and gained residence in endolysosomes (Ewald and others 2008; Park and others 2008; Chockalingam and others 2009). Moreover, trafficking of endosomal TLRs can be induced by stimuli other than nucleic acids, such as LPS (Blasius and Beutler 2010).

Several ER-associated proteins have been shown to act as chaperones and to mediate translocation of TLRs to endolysosomes and/or to the cell membrane (Fig. 2). GP96 (an ER paralog of the HSP90 family) affects trafficking of several TLRs, as macrophages deficient in this molecule do not respond to ligands for TLRs 1, 2, 4, 5, 7, or 9 (Randow and Seed 2001; Yang and others 2007a). Trafficking of TLRs 1, 2, and 4 from the ER to the plasma membrane, and TLRs 7 and 9 (but not TLR3) to the endolysosomes, is also affected by PRAT4A (Takahashi and others 2007). Another ER-resident protein central to the trafficking of TLRs 3, 7, and 9 to endolysosomes is Unc93b1. The 3d missense mutation of the *Unc93b1* gene, resulting in an H412R substitution in the ninth membrane-spanning region of the encoded protein, causes absence of signaling by TLRs 3, 7, and 9 and susceptibility of the mutant mice to infection by various pathogens (Tabeta and others 2006). Susceptibility to herpes simplex encephalitis was also observed in patients with *UNC93B1* gene mutations (Casrouge and others 2006). Further studies showed that the endosomal TLRs bind to the Unc93b1 protein via

their transmembrane domains (Brinkmann and others 2007), and that TLRs 7 and 9, but not TLR3, compete for association with this trafficking molecule (Fukui and others 2009). Interestingly, TLR9 binds to Unc93b1 more efficiently than TLR7, thus favoring its translocation to endolysosomes (Fukui and others 2009). Accordingly, TLR9 signaling is normally stronger than that of TLR7, and experimental TLR9 overexpression inhibits TLR7 signaling (Wang and others 2006). The preferential interaction with TLR9 is mediated by the N-terminal domain of Unc93b1, and when this domain is mutated (D34A), then the interaction favors TLR7 (Fukui and others 2009).

More recently, an additional set of proteins was shown to play a central role in the trafficking and signaling by nucleic acid-sensing TLRs and production of proinflammatory cytokines. It has long been recognized that signaling through TLR7 and TLR9 can trigger the production of TNF α , IL-12, IL-6, and pro-IL-1 β by activating NF- κ B and type I IFNs by activating IRF7. A recent study suggested that these two pathways are initiated from distinct cellular compartments (Fig. 2). A key molecule in this trafficking process appears to be AP-3, a 4-subunit (δ , β 3A, μ 3A, σ 3) clathrin-associated adaptor protein complex that recognizes dileucine-based motifs in transmembrane proteins and sorts these proteins to endosomes, lysosomes, or lysosome-related organelles (LRO) (Mattera and others 2011). Thus, pDCs and cDCs from AP-3-deficient mice (*Ap3b1*^{-/-}, lacking the β 3A subunit), as well as pDCs from mice with mutations in the β 3A or δ subunits of the AP-3 complex (*pearl* and *mocha* mice, respectively), showed defective signaling and production of type I IFNs in response to TLR7 or TLR9 agonists, but NF- κ B-mediated induction of IL-12p40 was unaffected (Sasai and others 2010). Further work showed that AP-3 is required for trafficking of TLR9 and Unc93b1 to late endosomes and LROs expressing the lysosomal-associated membrane protein 2 (LAMP2) marker, but not to early endosomes that express vesicle-associated membrane protein 3 (VAMP3). These and

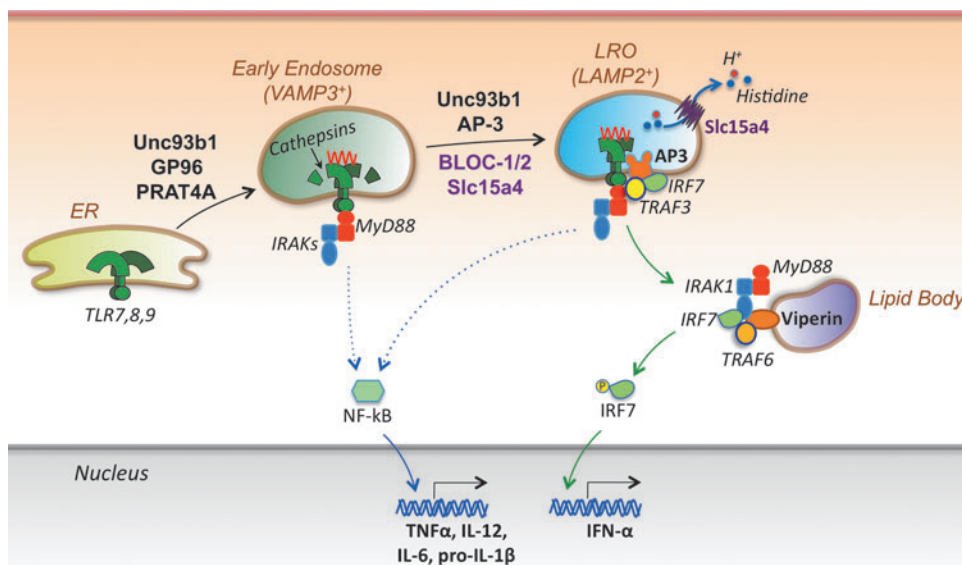


FIG. 2. TLR compartmentalization and trafficking. Nucleic acid-specific TLRs traffic from the endoplasmic reticulum (ER) to endolysosomes, where they undergo ligand binding and cathepsin-mediated proteolytic cleavage, a process required for efficient signaling. Several proteins have been shown to facilitate TLR translocation to endolysosomes, including the heat-shock protein gp96, PRAT4A, and Unc93b1. Moreover, type I IFN production by pDCs is strictly dependent on TLR localization to specialized compartments termed lysosome-related organelles (LROs). AP-3 (likely together with Unc93b1) mediates TLR translocation to LROs, whereas

other molecules in these organelles are required for effective type I IFN induction, including BLOC-1, BLOC-2, and Slc15a4 (a transporter of protons and histidines from the LRO lumen to the cytosol). In addition, the IFN-induced molecule viperin has been shown to localize to lipid bodies and promote assembly of a signaling complex that includes MyD88, IRAK1, and TRAF6, thereby greatly facilitating IRF7 activation and IFN- α production. One study suggests that IRF7 and NF- κ B signaling is initiated from LROs and early endosomes, respectively, whereas another study indicates that both pathways are initiated in the LROs.

additional experiments led to the formulation of a TLR bifurcation signaling model. According to this model, intracellular TLRs, together with Unc93b1, transit first to early endosomes in an AP-3-independent manner, resulting in NF- κ B-mediated production of proinflammatory cytokines (NF- κ B endosome), and then to late endosomes in an AP-3-dependent manner, thus resulting in TRAF3-mediated attraction and activation of IRF7 and production of type I IFNs (IRF7 endosome or LRO) (Fig. 2). This model suggests that nucleic acid-sensing TLRs, by trafficking into specialized cellular compartments, engage distinct pathways that lead to different cytokine profiles, presumably due to restricted localization of specific signal transduction molecules. It should be noted, however, that in another study with *Ap3b1* mutant mice (*pearl* and *bullet gray*) and highly purified DC subsets, the defective response to TLR9 engagement was detected in pDCs, but not cDCs, and production of both type I IFNs and TNF- α was reduced (Blasius and others 2010). Thus, rather than a bifurcation model, this latter study suggests that in pDCs, both NF- κ B and IRF7 signaling pathways are initiated from a specific LRO present in this cell type.

Of interest, mutations in the AP-3 complex have been linked to the Hermansky–Pudlak syndrome, which defines a group of human autosomal recessive disorders characterized by abnormal biogenesis and function of several types of LROs, such as melanosomes, platelet-dense granules, lamellar bodies of type II alveolar epithelial cells, and lytic granules of cytotoxic T cells and NK cells (Dell'Angelica 2009). The clinical phenotype of this syndrome is characterized by grades of oculocutaneous albinism, a bleeding diathesis due to platelet defects, T lymphocyte dysfunction, and neutropenia. Mutations affecting three ubiquitously expressed protein complexes, named biogenesis of lysosome-related organelles complex (BLOC) -1, -2, and -3, are also associated with Hermansky–Pudlak syndrome (Dell'Angelica 2004). Accordingly, mice carrying the *salt and pepper* mutation in the *Dtnbp1* gene (encoding dysbindin, a component of BLOC-1), or the *toffee* mutation in the *Hps5* gene (encoding a component of BLOC-2), exhibited defective type I IFN responses of pDCs to a TLR9 ligand (Blasius and others 2010). Interestingly, the AP-3 complex interacts via its μ subunit with dysbindin in the BLOC-1 complex (Taneichi-Kuroda and others 2009), and this interaction may be related to the role of both AP-3 and BLOC-1 in endosomal TLR signaling and type I IFN production by pDCs.

Another mutant mouse, *feeble*, with defective production of type I IFNs as well as TNF- α , IL-6, and IL-12p40 after TLR7 or TLR9 ligation, has been identified (Blasius and others 2010). This defect was confined to pDCs, but the development of these cells was unaffected. The phenotype of these mice was mapped to a mutation in the *Slc15a4* gene, which encodes the proton/histidine transporter 1 (PHT1). The solute carrier subfamily 15 (Slc15) contains 4 members (Slc15a1 to Slc15a4) and is a part of the proton-coupled oligopeptide transporter superfamily (Nielsen and Brodin 2003; Daniel and Kottra 2004). Of these, Slc15a1 (also called PEPT1) and Slc15a2 (PEPT2) have broad substrate specificity and transport a large spectrum of di- and tri-peptides, whereas Slc15a3 (PHT2) and Slc15a4 (PHT1) are more specific in that their function is primarily to transport free histidine and certain oligopeptides from inside the endosome to the cytosol (Yamashita and others 1997; Bhardwaj and others 2006). The transport activity of Slc15a4 is pH-dependent,

with a higher transport of histidine at pH 5.5 than at pH 7.0 (Yamashita and others 1997), thereby suggesting that the function of this transporter is related to endosomal acidification and lysosomal maturation. Interestingly, Slc15a4 contains a dileucine motif in its N-terminal region that might be recognized by AP-3, thereby suggesting comigration of these two molecules to the endolysosomes. The findings overall suggest that AP-3 primarily affects TLR trafficking, whereas Slc15a4 may be required to create an endolysosomal microenvironment optimal for TLR activation and/or signaling.

The special role of certain organelles in the activation of endosomal TLRs has further been documented by the finding that Viperin, a protein induced by IFNs and even directly by viruses and bacteria (Fitzgerald 2011), is required for TLR7- and TLR9-mediated production of type I IFNs by pDCs (Jiang and Chen 2011; Saitoh and others 2011). Viperin, however, had no role in the production of other inflammatory cytokines (IL-12, TNF- α , and IL-1 β) by pDCs and other cell types, nor in the production of type I IFNs by cDCs, macrophages, and fibroblasts, or signaling by other sensors (TLR4 and RLRs). Mechanistically, it was shown that an amphipathic α -helix in the N-terminus of Viperin targets this protein to the cytosolic surface of ER-derived lipid storage organelles, called lipid bodies. These organelles appear to function downstream of the LROs with Viperin serving as a platform for the attraction of signaling effectors, including MyD88, TRAF6, IRAK1, and IRF7. Collectively, the findings with molecules that affect formation and function of specific subcellular compartments, such as AP-3, BLOCs, Slc15a4, and Viperin, have provided novel clues for elucidating the reason that pDCs are such prodigious producers of type I IFNs.

Endosomal TLR activation requires processing by proteases

Activation of nucleic acid-specific TLRs is confined to endolysosomes, thereby precluding their engagement by self-nucleic acids and ensuring that responses are initiated after the breakdown of pathogens in this acidified microenvironment. Recent studies have shown that functionality of these receptors, but not ligand binding, is only acquired in endolysosomes on cleavage of a major portion of the ectodomain by resident acid-dependent proteases, principally cathepsins (Ewald and others 2008; Matsumoto and others 2008; Park and others 2008). Individual cathepsins and/or combinations thereof are required for activation of all endosomal TLRs, and this requirement is conserved across all cell types (Ewald and others 2011). The asparagine endopeptidase (AEP) legumain was also shown to participate in TLR cleavage (Sepulveda and others 2009; Ewald and others 2011). Overall, TLR processing appears to proceed through a two-step mechanism, ie, cleavage of a portion of the ectodomain mediated by multiple cathepsins (perhaps with some participation of legumain) followed by N-terminal trimming (Ewald and others 2011). The reason that ectodomain cleavage is required for MyD88 recruitment and signaling by endosomal TLRs remains to be clarified. However, conformational changes induced by this process may facilitate TLR dimerization, an event critical for efficient signaling (Latz and others 2007). The finding that endosomal TLR activation requires cathepsin-mediated processing explains

previous observations in mouse models that pharmacologic inhibition or deletion of cathepsins potently suppressed joint inflammation (Asagiri and others 2008), neuroinflammation (Asagiri and others 2008), autoimmune diabetes (Maehr and others 2005; Hsing and others 2010), and Sjögren's syndrome-like manifestations (Saegusa and others 2002).

Cytosolic Nucleic Acid Sensors

There is also a broad spectrum of receptors that evolved to recognize nucleic acids in the cytosol, and their signaling is critical for the initiation of innate responses against certain microbes. Two main classes of such sensors have been identified, one specific for RNA and the other for DNA. Most of these sensors activate transcription factors leading to expression of type I IFNs and other cytokines, but a few may promote the assembly of an inflammasome and secretion of IL-1 β .

RNA sensors

The RNA sensors encompass three helicases, RIG-I, melanoma differentiation-associated gene 5 (MDA5, also known as IFN-induced with helicase C domain 1, IFIH1), and laboratory of genetics and physiology 2 (LGP2) specific for distinct but overlapping sets of viruses (Moore and Ting 2008; Takeuchi and Akira 2008; Matsumiya and Stafforini

2010) (Fig. 3). RIG-I recognizes ssRNA and dsRNA with uncapped 5'-triphosphate ends as well as short dsRNA structures (even without 5'-triphosphates), whereas MDA5 recognizes long dsRNA species with blunt ends (Pichlmair and others 2006; Schlee and others 2009; Yoneyama and Fujita 2009). In addition to a central DEAD box helicase/ATPase domain, RIG-I and MDA5 also display tandem N-terminal caspase activation and recruitment domains (CARD), and a C-terminal domain (CTD) containing a repressor domain. In resting cells, RIG-I is maintained as a monomer in a latent autoinhibited, "closed" conformation state. After binding of CTD to the 5'-triphosphate moiety of RNA, ATP hydrolysis by the helicase domain results in a conformational change that displaces the CTD and exposes the CARDs, which, through a homotypic interaction, engage the mitochondria-anchored adaptor IFN β promoter stimulator-1 (IPS-1) (also known as mitochondrial antiviral signaling protein [MAVS], CARD-containing adaptor protein [CARDIF], and virus-induced signaling adaptor [VISA]). This engagement is associated with redistribution of IPS-1 to form speckle-like aggregates, a process mediated by Mitofusin 1 and 2 (Onoguchi and others 2010; Koshiba and others 2011), followed by recruitment of TBK1 and other signaling molecules that drive expression of type I IFNs. IPS-1 was also shown to be present on the surface of peroxisomes, organelles that, in concert with mitochondria, affect metabolism of

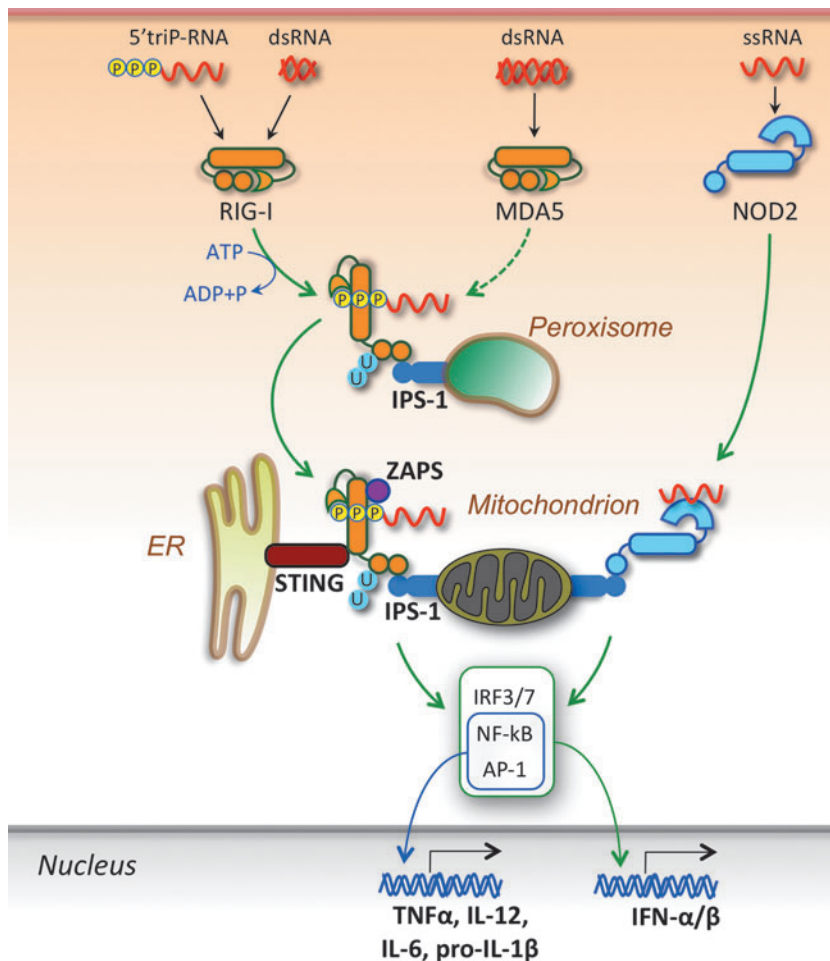


FIG. 3. Cytosolic RNA sensors. RNA in the cytoplasm is primarily sensed by the helicases RIG-I (RNA with uncapped 5'-triphosphates or short dsRNA) and MDA5 (long dsRNA). Ligand binding induces ATP-dependent conformational changes that allow CARD/CARD homotypic interactions between these helicases and the signaling adaptor IPS-1 localized on either peroxisomes or mitochondria. Engagement of peroxisomal IPS-1 promotes a transient IFN-independent response, whereas at the mitochondrial membrane, the engaged IPS-1 relays a signaling cascade that leads to transcription factor activation and expression of type I IFNs and proinflammatory cytokines. RIG-I signaling also requires the participation of the ER-associated STING and is enhanced by the interaction with ZAPS as well as by TRIM25-mediated K63-linked ubiquitination (U) of CARDs, although unanchored ubiquitin chains also promote RIG-I activation. Another helicase, LGP2, lacks CARDs and appears to facilitate RNA binding to RIG-I and MDA5. In addition, NOD2, a CARD-containing member of the NLR family mostly dedicated to the detection of the peptidoglycan component muramyl dipeptide (MDP) found in both Gram-positive and Gram-negative bacteria, can also recognize ssRNA in the cytosol and promote type I IFN and proinflammatory cytokine production through an IPS-1-mediated pathway.

lipids and reactive oxygen species (Dixit and others 2010). Engagement of peroxisomal IPS-1 precedes that of mitochondrial IPS-1, is short lived, and initiates an early IFN-independent antiviral response that is then potentiated by the mitochondrial IPS-1-induced type I IFN response. Post-translational modifications affect the RIG-I/IPS-1 pathway, with ubiquitination of CARDs by tripartite motif protein 25 (TRIM25) promoting signaling (Yoneyama and Fujita 2009). In this regard, a remarkable finding was that unanchored ubiquitin chains (not conjugated to any target molecule) together with RNA potently activate RIG-I (Zeng and others 2010). The third helicase, LGP2, does not contain CARDs required for signaling, but rather facilitates viral RNA recognition by RIG-I and MDA5, acting either directly by altering RNA conformation or indirectly by dislodging viral ribonucleoproteins (RNPs) in an ATP-dependent manner to expose RNA (Moresco and Beutler 2010; Satoh and others 2010).

An ER- and mitochondria-associated molecule, stimulator of IFN genes (STING, also known as MITA, MPYS, TMEM173 and ERIS), appears to be essential for efficient type I IFN induction by RIG-I, but not MDA5, and likely acts as an anchor that facilitates the interaction of the sensor with downstream signaling molecules (Ishikawa and Barber 2008; Zhong and others 2008; Ishikawa and others 2009; Barber 2011). In addition, signaling strength and duration are enhanced by the direct interaction of RIG-I with signaling cofactors, including the zinc-finger antiviral protein shorter isoform (ZAPS), a member of the poly(ADP-ribose) polymerase family (Hayakawa and others 2011; Liu and Gale 2011), and the dsRNA-binding protein PACT (Kok and others 2011). A recent study also showed that the protein kinase R regulates the integrity of IFN- β transcripts induced by several MDA5-dependent viruses (Schulz and others 2010).

Interestingly, NOD2, a CARD-containing member of the large NLR family of cytosolic sensors that principally recognizes muramyl dipeptides (MDPs) found in nearly all Gram-positive and Gram-negative bacteria, was also reported to recognize viral ssRNA (Sabbah and others 2009). In fact, NOD2 recognition of MDP leads to NF- κ B activation and induction of proinflammatory cytokines, whereas recognition of ssRNA promotes IPS-1-dependent IRF3 activation and type I IFN production. This is an interesting example of a receptor that recognizes two structurally distinct ligands causing induction of alternative signaling pathways, and poses questions about the presumed strict specificity of innate sensors for certain molecular signatures.

Another member of the NLR family, NLRX1, has also been shown to interact with IPS-1, but this interaction leads to inhibition of RIG-I-mediated IFN- β production, thus suggesting that some NLRs might function as modulators of pathogen responses rather than as classical sensors (Moore and others 2008; Allen and others 2011; Xia and others 2011). Other cellular regulators that suppress RLR signaling include the deubiquitinating enzyme A and cylindromatosis tumor suppressor (CYLD), the ubiquitin-ligase RNF125, the autophagy-related Atg5-Atg12 conjugate, and caspase-8 (Gack and others 2008; Moore and Ting 2008; Oshiumi and others 2009; Liu and Gu 2011; Rajput and others 2011). In addition, the intracellular form of the klotho protein has been shown to inhibit the RIG-I-mediated expression of IL-6 and IL-8 induced in senescent cells through the ataxia telangiectasia mutated-IRF1 axis (Liu and others 2011a). As in the case of

TLRs, viruses also have acquired several mechanisms to evade and/or suppress production of type I IFNs by the RLR pathway, including expression of proteins that inhibit helicase-ligand interactions or proteases that cleave IPS-1 from the mitochondrion (Takeuchi and Akira 2008).

DNA sensors

Cytosolic dsDNA, particularly of large size, is recognized by a wide spectrum of sensors, thus leading to activation of diverse signaling pathways (Fig. 4). Critically, recognition of cytosolic DNA appears to be sequence independent and largely unaffected by the degree of methylation, thus suggesting that both foreign and self-DNA may initiate such responses. Early studies showed that intracellular administration of the right-handed B-form DNA or a synthetic 45-mer DNA of random sequence lacking CpG motifs triggered type I IFN production in a TLR-independent manner (Ishii and others 2006; Stetson and Medzhitov 2006). A sensor termed DNA-dependent activator of IRFs (DAI, also known as Z-DNA binding protein-1 [ZBP-1] and DLM-1), was subsequently reported to recognize both the B-form and the left-handed Z-form of cytosolic dsDNA and to induce type I IFNs (Takaoka and others 2007). However, this response was not compromised in cells derived from DAI-deficient mice (Ishii and others 2008), thus implying the existence of additional sensors or a cell-specific role for these sensors (Wang and others 2008; DeFilippis and others 2010).

Another means of cytosolic DNA recognition is mediated by DNA-dependent RNA polymerase III. This polymerase transcribes AT-rich dsDNA in a promoter-independent manner to generate dsRNA intermediates bearing uncapped 5'-triphosphates that serve as agonists for RIG-I/IPS-1-dependent type I IFN production, a response that, in part, requires the participation of STING (Ablasser and others 2009; Chiu and others 2009; Barber 2011).

Additional studies showed that interaction with cytosolic DNA is also mediated by human IFI16 and its mouse ortholog p204, which are pyrin domain (PYD)-containing members of the hematopoietic IFN-inducible nuclear (HIN-200) family of proteins (PYHIN) (Unterholzner and others 2010). Although IFI16 is mostly localized in the nucleus, small amounts of this factor might egress into the cytoplasm where recognition of DNA and downstream signaling events lead to type I IFN production. Alternatively, IFI16 recognition of viral DNA may take place in the nucleus followed by migration to the cytoplasm to stimulate signal transduction, as suggested by studies with herpes simplex virus-1 (HSV-1) (Unterholzner and others 2010). As is the case for RIG-I and RNA polymerase III, IFI16 signaling also requires the participation of STING (Unterholzner and others 2010). With regard to the mechanism by which STING contributes to the signaling induced by cytosolic DNA, it has been shown that, on stimulation, STING translocates from the ER to Golgi and finally to cytoplasmic punctate structures to assemble with TBK1, a process that appears to be negatively regulated by the autophagy-related gene 9a (Saitoh and others 2009; Barber 2011).

Four other sensors for cytosolic nucleic acids with unique properties, DHX9, DHX36, LRRFIP1, and Ku70, have been identified. DHX9 and DHX36 are members of the DEXD/H box family of helicases that also includes RLRs. They recognize CpG-DNA in pDCs and mediate MyD88-dependent

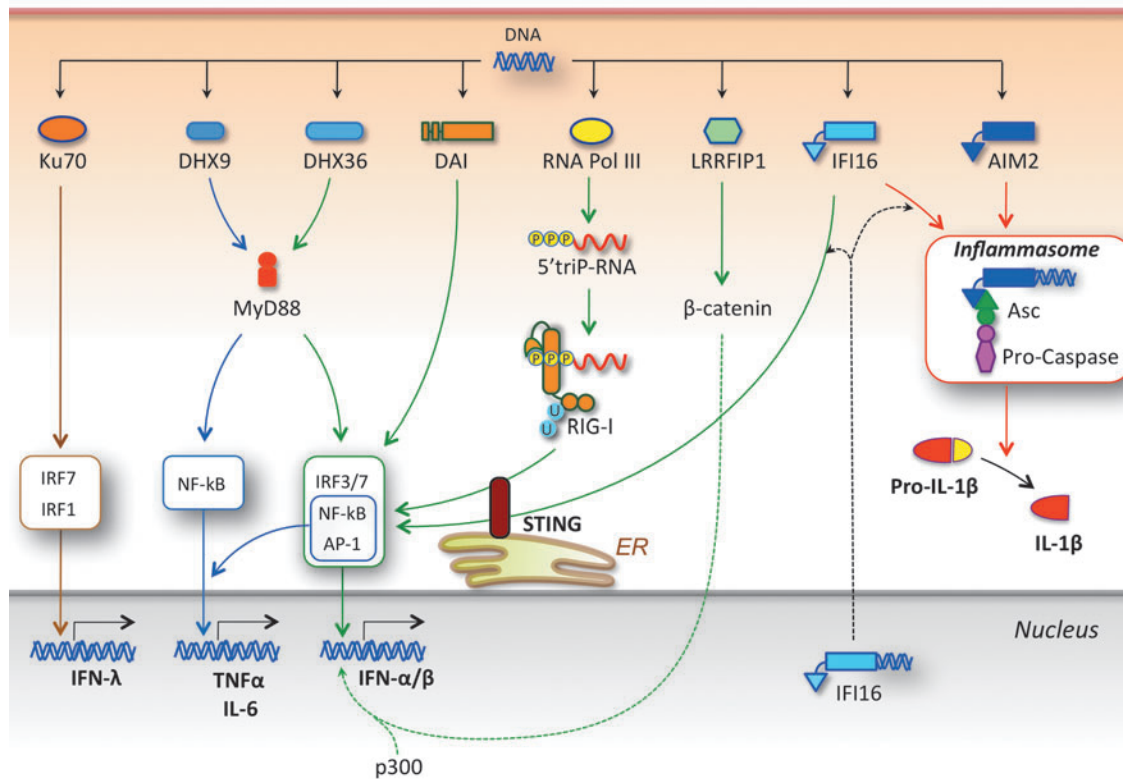


FIG. 4. Cytosolic DNA sensors. A large panel of sensors is dedicated to the detection of DNA in the cytosol. Among them, Ku70 induces IFN-λ, DHX9 induces proinflammatory cytokines, and DHX36, DAI, RNA polymerase III, and IFI16 induce production of type I IFNs and proinflammatory cytokines. RNA polymerase III acts by transcribing DNA into RNA molecules bearing uncapped 5'-triphosphates, which bind RIG-I and engage the IPS-1/STING pathway (see Fig. 3). Another sensor, LRRFIP1, binds DNA (or dsRNA) and activates β-catenin, which migrates to the nucleus and potentiates *IFNB* gene transcription by promoting recruitment of the acetyltransferase p300 to the IFN enhanceosome. In addition, IFI16 and AIM2 induce the assembly of an inflammasome by recruiting pro-caspases via the adaptor ASC, thus leading to caspase-mediated activation and secretion of IL-1β and IL-18. IFI16 (and perhaps other sensors) may recognize DNA in the nucleus and then migrate to the cytoplasm to initiate signal activation.

induction of proinflammatory cytokines (via NF-κB) and type I IFNs (via IRF7), respectively (Kim and others 2010). LRRFIP1 notably recognizes both dsRNA and dsDNA and, by activating β-catenin, facilitates recruitment of the acetyltransferase p300 to the IFN enhanceosome, which potentiates *IFNB* gene transcription (Yang and others 2010). On the other hand, Ku70, a component of the heterodimeric Ku protein required for end-joining DNA repair, VDJ recombination, telomere maintenance, and other nuclear processes, recognizes various types of cytosolic DNA and promotes production of the type III IFN (IFN-λ1) through activation of IRF1 and IRF7 (Zhang and others 2011).

Inflammasome induction by cytosolic nucleic acids

Previous studies noted that intracellular bacterial, viral, and mammalian dsDNA could trigger the production of the potent proinflammatory cytokine IL-1β in a cell-specific manner, thus suggesting the involvement of an inflammasome (Muruve and others 2008). The sensor involved in this response was identified as absent in melanoma 2 (AIM2), a cytoplasmic member of the PYHIN family (Burckstummer and others 2009; Fernandes-Alnemri and others 2009; Hornung and others 2009; Roberts and others 2009). AIM2 binds DNA via its HIN-200 domain, recruits the adaptor apoptosis-

associated speck-like protein containing a CARD (ASC) via PYD-PYD interactions, and then ASC attracts pro-caspase-1 through CARD-CARD interactions. On autoactivation, caspase-1 cleaves pro-IL-1β (and pro-IL-18) to a mature secreted form. The AIM2 inflammasome has also been reported to induce a specific form of cell death termed pyroptosis that involves DNA damage but, unlike apoptosis, is associated with a loss of plasma membrane integrity and maintenance of mitochondrial membrane potential (Fernandes-Alnemri and others 2009).

In addition to type I IFN-induction (see above), IFI16 can also mediate the assembly of an ASC-dependent inflammasome in the cytosol after recognition of Kaposi Sarcoma-associated herpes virus (KSHV) in the nucleus (Kerur and others 2011). The findings on nuclear recognition of HSV-1 and KSHV by IFI16 raise the question of how engagement of this sensor by self-nucleic acids is avoided, and masking of cellular DNA by histones and other proteins or recognition restricted for damaged DNA might be potential explanations (Unterholzner and Bowie 2011). Interestingly, in a manner similar to IFI16, the RNA sensor RIG-I has also been shown to induce both type I IFN production and the assembly of an inflammasome, the latter through the recruitment of ASC and pro-caspases 1 and 3 (Kim and Yoo 2008; Rintahaka and others 2008; Poeck and others 2010).

Nucleic Acid Sensors and Autoimmunity

Shortly after or concurrent with the discovery of nucleic acid sensors, an extensive body of evidence has been gathered to suggest that these sensors and the resulting induction of proinflammatory cytokines play an important effector role in the pathogenesis of autoimmune diseases, with the bulk of the evidence, at present, related to endosomal TLRs and systemic autoimmunity.

Endosomal TLRs and systemic autoimmunity

As discussed, specific mechanisms have evolved to prevent the engagement of intracellular innate sensors by self-nucleic acids, including compartmentalization of functional TLRs to endolysosomes, rarity and/or chemical modification of stimulatory motifs in mammalian nucleic acids (eg, methylation, capping of 5'-triphosphates of RNA molecules), and clearance of self-nucleic acids and apoptotic/necrotic materials containing such ligands by nucleases and other processes. However, several pathways have been identified by which these barriers can be overcome, thus leading to pathological consequences, particularly in lupus (Fig. 5). Specifically with regard to nucleic acid-sensing TLRs, these pathways involve autoantibodies bound to subcellular particles containing self-nucleic acids, which are taken up by pDCs via FcR, or by B cells via BCRs specific for either antigenic determinants in the complex or the Fc portion of the autoantibodies (Ronnlblom and others 2006; Marshak-Rothstein and Rifkin 2007; Theofilopoulos and others 2010).

The synergistic engagement of BCR and TLR induces enhanced B cell proliferation, whereas production of type I IFN by pDCs leads to B cell differentiation and immunoglobulin isotype switching. Free nucleic acids or particles containing such molecules (ie, nucleosomes, RNPs) can also interact with specific BCRs (Viglianti and others 2003), and recent evidence suggests that BCR signaling induces fusion of TLR9-containing endosomes with the internalized BCR into autophagosomes, thereby facilitating B cell activation (Charurvedi and others 2008; Monroe and Keir 2008).

The uptake of nucleic acids and related complexes by pDCs and B cells is enhanced by certain accessory molecules, such as the high-mobility group box (HMGB) proteins and the antimicrobial peptide LL37. HMGB proteins are highly expressed in the nucleus, where they regulate chromatin structure and transcription, but they are also present in the cytosol and are released from necrotic cells and cells stimulated with TLR ligands or cytokines, thus potentially inducing inflammatory responses (Scaffidi and others 2002; Bianchi and Manfredi 2007). Initial studies showed that HMGB1 enhanced TLR9-mediated signaling in response to CpG-DNA or nucleic acid-containing complexes, likely by facilitating ligand uptake either directly or through the receptor for advanced glycation end-products (RAGE) (Ivanov and others 2007; Tian and others 2007). Interestingly, DNA-C3a complexes were also reported to stimulate type I IFN production in a RAGE-dependent manner (Ruan and others 2010). Moreover, increased levels of HMGB1 in serum of patients with lupus have been shown to correlate with

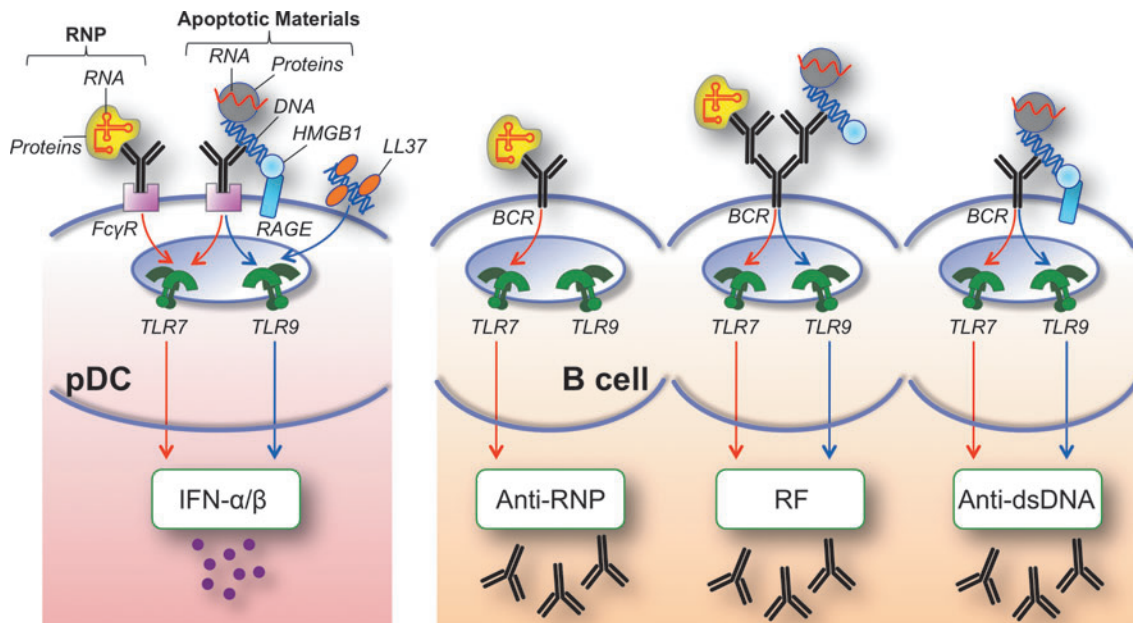


FIG. 5. Mechanisms by which barriers for TLR recognition of self-nucleic acids are breached in systemic autoimmunity. Various mechanisms usually prevent TLR engagement by self-nucleic acids, including nuclease-mediated degradation, exclusion from endosomes, and methylation. Autoantibodies and BCRs specific for nucleic acid-containing particles (eg, anti-RNP, anti-DNA) or exhibiting rheumatoid factor activity (RF) can overcome these mechanisms, mediating nucleic acid delivery to endosomes in pDCs (via FcγR) and B cells (via BCR), and promoting type I IFN (IFN-α/β) and autoantibody production. Nucleic acid-binding accessory proteins such as HMGB1 (in part via RAGE) and LL37 can also facilitate DNA uptake and TLR engagement. Production of anti-RNP autoantibodies requires TLR7, whereas production of anti-dsDNA and RF autoantibodies requires either TLR7 or TLR9. Thus, the major antigen for autoantibodies to RNP contains RNA (red) and proteins (particle in yellow), whereas the antigenic target of anti-DNA autoantibodies contains DNA (blue), RNA, and other accessory molecules (particle in gray).

disease activity (Andersson and Rauvala 2011; Urbanaviciute and Voll 2011). Strikingly, a more recent study expanded the role of HMGB proteins by demonstrating that HMGB1, HMGB2, and HMGB3 act as universal sentinels for both DNA and RNA and that their presence is required for efficient signaling by cytosolic and endolysosomal nucleic acid sensors (Yanai and others 2009; Yanai and others 2011). The exact mechanism by which HMGB proteins deliver nucleic acids to these sensors and the circumstances under which this function may exert beneficial or detrimental effects to the organism remain to be elucidated.

DNA and RNA delivery to endosomes leading to TLR engagement in pDCs, myeloid DCs, and B cells are also promoted by the nucleic acid-binding LL37 cathelicidin, a mammalian antimicrobial amphipathic peptide (Lande and others 2007; Ganguly and others 2009; Hurtado and Peh 2010). LL37 is produced by keratinocytes and released after skin injury, and this response may be involved in the pathogenesis of psoriasis (Lande and others 2007). However, other studies have presented evidence that the Aim2/DNA inflammasome may be involved in psoriasis, and that LL37 induced by vitamin D may inhibit this inflammasome (Dombrowski and others 2011). This finding suggests a potential beneficial role of LL37 and is consonant with the known therapeutic effects of vitamin D in this condition. Whether LL37 or Aim2 have any relevance to dermatologic manifestations of lupus, particularly after UV exposure, remains to be addressed.

Several studies in humans and mice have demonstrated the primary role of endosomal TLR engagement and type I IFN production in the pathogenesis of lupus (Theofilopoulos and others 2010). In SLE, there is an "IFN signature" (a dominance of genes affected by type I IFNs) that appears to correlate with clinical severity (Baechler and others 2004; Kirou and others 2005; Banchereau and Pascual 2006; Nikpour and others 2008; Petri and others 2009; Obermoser and Pascual 2010). Polymorphic variants of TLR7 that may associate with increased TLR7 transcripts and more pronounced IFN signatures were reported to constitute a predisposing factor in Eastern Asian patients with lupus (Shen and others 2010; Kawasaki and others 2011), and greater *TLR7* gene copy number increased risk of SLE in the Mexican population (Garcia-Ortiz and others 2010). However, other studies found no detectable association between lupus and *TLR7* gene copy number or polymorphic variants (Demirci and others 2007; Kelley and others 2007; Sanchez and others 2009), thus suggesting contributions by additional genetic factors. Genome-wide and other studies have also revealed an association of SLE susceptibility with genetic variants of IRF5, IRF7, IRAK1, STAT4, and Tyk2, molecules involved in type I IFN signaling pathways (Harley and others 2009; Fu and others 2011). Interestingly, underexpression of the microRNA miR-146a, a negative regulator of the IFN pathway, correlated with disease activity in patients with SLE (Tang and others 2009), and targeted deletion of this microRNA in mice was associated with some lupus-like manifestations (Boldin and others 2011). Expression and/or function of several other microRNAs have been implicated in SLE and mouse models, but the exact mechanisms of action and whether they affect innate responses and cytokine production remain to be clarified (Pauley and others 2009; Vinuesa and others 2009; Xiao and Rajewsky 2009; Dai and others 2010).

The pathogenic role of endosomal TLRs and type I IFNs has been directly documented in the classical spontaneous lupus strains of mice. Previous studies showed that NZB mice deleted of the common receptor for type I IFNs had significantly reduced disease (Santiago-Raber and others 2003), whereas, conversely, administration of recombinant IFN- α , plasmids encoding this cytokine or synthetic endosomal TLR ligands exacerbated disease in NZBxW, NZBxBXSB, NZM2328, and B6.Sle123 mice (Mathian and others 2005; Fairhurst and others 2008; Jacob and others 2011; Ramanujam and others 2009; Triantafyllopoulou and others 2010). Moreover, *MRL-Fas^{lpr}* deficient in IRF5 showed decreased serologic, cellular, and histologic disease characteristics and increased survival (Tada and others 2011). A recent study showed that treatment of NZBxW mice with IFN- α led to the induction of short-lived, but not long-lived, plasma cells, presumably due to changes in the niches that sustain plasma cell survival (Mathian and others 2011), whereas a proteasome inhibitor (bortezomib) that depletes both short- and long-lived plasma cells protected lupus-predisposed mice (Neubert and others 2008). Interestingly, bortezomib was also shown to suppress function and survival of pDCs by disrupting the coordinated translocation of TLRs and Unc93b1 and disturbing ER homeostasis (Hirai and others 2011). IFN- α -driven autoantibody production and nephritis in NZBxW mice was dependent on CD4 T cell help (Liu and others 2011c), as expected based on the fact that contributions by both innate and adaptive systems are required for full disease expression (Baccala and others 2007).

The strongest evidence for the role of endosomal TLRs in systemic autoimmunity was obtained in male BXSB mice bearing the Y-linked autoimmune accelerating locus (Yaa), where a translocation from the X to Y chromosome leads to the duplication of several genes, including *Tlr7* (Pisitkun and others 2006; Subramanian and others 2006). The principal role of the *Tlr7* gene duplication was shown by significant disease reduction in male BXSB mice carrying half of the *Tlr7* gene dosage (due to *Tlr7* null mutation in the X chromosome) and, conversely, the appearance of autoimmune manifestations in transgenic normal background mice with increased copy numbers of the *Tlr7* gene (Deane and others 2007). However, *Tlr7* duplication in itself is insufficient but rather, as expected in this polygenic disorder, requires additional genetic contributions, as demonstrated by absence of disease in normal background mice consomic for the Yaa chromosome (Theofilopoulos and Dixon 1985). Moreover, genes other than *Tlr7* in the duplicated segment also appear to contribute, as introduction of a *Tlr7* null mutation on the X chromosome of C57BL/6.Nba2 mice congenic for the Yaa chromosome led to incomplete disease resolution (Santiago-Raber and others 2008). *MRL-Fas^{lpr}* mice lacking TLR7 also showed considerable decreases in the levels of anti-Sm autoantibodies and a modest reduction of renal disease (Christensen and others 2006). Further documentation for the central role of nucleic acid-specific TLRs in murine lupus was obtained with BXSB and C57BL/6-*Fas^{lpr}* mice congenic for the *Unc93b1 3d* mutation, which extinguishes signaling through TLRs 3, 7, and 9 (Kono and others 2009). Interestingly, patients deficient for UNC93B1, despite showing defective central and peripheral B cell tolerance and accumulation of large numbers of autoreactive mature B cells in their blood, did not develop autoantibodies or histologic

autoimmune manifestations, thus further documenting the central role of UNC93B1 and endosomal TLRs in promoting systemic autoimmunity (Isnardi and others 2008).

However, there is considerable controversy with regard to the role of TLR9 in lupus. This is because, paradoxically, deficiency of TLR9 caused an overall disease enhancement in the apoptosis-defective MRL-*Fas*^{lpr} mice, despite reductions in anti-dsDNA autoantibody titers (Christensen and others 2005; Christensen and others 2006; Wu and Peng 2006). This finding parallels that in TLR8-deficient C57BL/6 mice, which developed lupus-like serologic and histopathologic characteristics (Demaria and others 2010). Interestingly, in both TLR9- and TLR8-deleted mice, DCs overexpressed TLR7 and showed stronger activation on stimulation with a TLR7 ligand; whereas combined deletion of TLR9 or TLR8 with TLR7 abolished autoimmunity (Demaria and others 2010; Santiago-Raber and others 2010). Moreover, the mutation D34A in the N-terminus of Unc93b1, which abolishes interaction with TLR9, rendered DC hyperresponsive to TLR7 (Fukui and others 2009), with a concomitant induction of systemic inflammation associated with expansion of Th1 and Th17 cells and various autoantibodies, including ANA (Fukui and others 2011).

The presumed protective effect of TLR9 contrasts with the following: (a) TLR9 engagement by DNA-containing immune complexes promoted B cell proliferation (Leadbetter and others 2002), (b) TLR7-deleted MRL-*Fas*^{lpr} mice showed considerably more residual disease than mice lacking both TLR7 and TLR9 (Nickerson and others 2010), and (c) disease was prevented in 3d mutant C57BL/6-*Fas*^{lpr} mice (Kono and others 2009). We, therefore, hypothesized that both TLRs exert an adverse effect, but the pathogenic contribution of TLR7 is stronger than that of TLR9 (Fig. 6). This hypothesis is supported by the finding that Unc93b1 mediates the trafficking of all nucleic acid-sensing TLRs to endolysosomes. Since TLR9 exhibits higher affinity for this molecule, it is reasonable to suggest that the absence of TLR9 will result in increased trafficking of TLR7 and enhanced disease, as, in fact, occurred in the examples just given. Although it is still unclear why TLR7 is more pathogenic than TLR9, likely possibilities include differences in signaling intensity and/or higher availability of particles that contain TLR7-engaging ligands such as snRNPs (Theofilopoulos and others 2010).

Thus, apart from minor incongruities, the published data strongly indicate that engagement of nucleic acid-specific TLRs is critical for the pathogenesis of lupus. The emerging

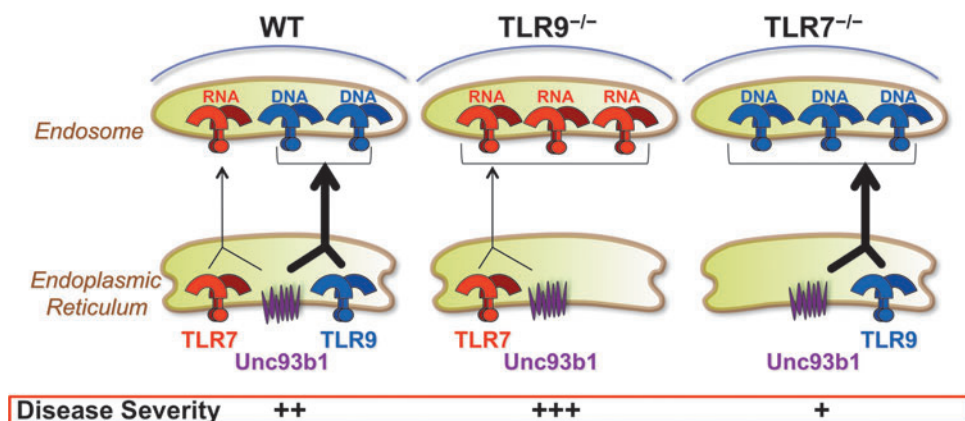
scenario is that signaling by these TLRs leads to the induction of proinflammatory cytokines, of which type I IFNs predominate. These pleiotropic cytokines induce maturation of DCs, upregulation of MHC and costimulatory molecules, production of B cell-trophic factors (B cell activating factor [BAFF]), and a proliferation-inducing ligand [APRIL]), and activation of previously quiescent autoreactive T and B cells. The outcome is production of autoantibodies and creation of immune complexes with particles containing nucleic acids, which, by engaging endosomal TLRs on several cellular targets (pDCs, DCs, and B cells), perpetuate and amplify the pathogenic autoimmune process. Type II IFN- γ , produced by innate and adaptive immune cells, also contributes to lupus, particularly at later stages of the disease, as suggested by increased IFN- γ levels in serum and IFN- γ -induced transcripts in PBMC of patients with SLE; disease inhibition in lupus-predisposed mice after deletion of IFN- γ or its receptor; and blockade of IFN- γ signaling by using antibodies, recombinant soluble receptors, or a plasmid encoding this receptor (Baccala and others 2005).

Although we focus here on the role of endosomal TLRs and type I IFNs in lupus, considerable evidence suggests that similar inflammatory pathways may also be involved in other autoimmune diseases, including rheumatoid arthritis, Sjögren's syndrome, type I diabetes, myasthenia gravis, hemolytic anemia, neuromyelitis optica, polymyositis/dermatomyositis, and psoriasis (Baccala and others 2005; Theofilopoulos and others 2005).

Cytosolic innate sensors and autoimmunity

Cytosolic nucleic acid sensors may also play a role in the pathogenesis of autoimmune diseases, but the evidence is mostly indirect. Nonetheless, incompletely digested DNA can provoke inflammatory responses, as inferred by a few patients with SLE with mutations in DNase I (Yasutomo and others 2001), lupus-like manifestations in mice deficient in DNase I (Napirei and others 2000), and anemia or arthritis in mice deficient in DNase II (Nagata 2008). DNase I is the major serum endonuclease that degrades extracellular dsDNA, whereas DNase II is localized in lysosomes and degrades chromosomal DNA from apoptotic cells and nuclei expelled from erythroid precursors. Moreover, mutations in the ER-localized 3' repair exonuclease 1 (Trex1, also known as DNase III) were found in a few patients with SLE (Lee-Kirsch and others 2007). In addition, mutations in Trex1 or

FIG. 6. Differential Unc93b1-mediated trafficking of TLR7 versus TLR9 may affect lupus pathogenesis in predisposed mice. Increased disease severity in TLR9-deleted lupus-predisposed mice may be due to increased availability of the common Unc93b1 trafficking partner, thus leading to enhanced translocation of the more pathogenic TLR7 to endolysosomes.



RNaseH2 have been associated with the Aicardi-Goutières syndrome and chilblain lupus, two conditions with some clinical and pathophysiological similarities to SLE (Crow and others 2006a; Crow and others 2006b; Lee-Kirsch and others 2007; Kavanagh and others 2008; Perrino and others 2009). Interestingly, autoimmunity was also observed in mice with Trex1-deficiency and attributed to accumulation of ssDNA in the ER (Yang and others 2007b), possibly derived from reverse transcription of endogenous retroelements (Stetson and others 2008). In all these conditions, disease is presumably mediated by type I IFN-inducing cytosolic sensors, as autoimmunity in Trex1-deficient mice was prevented by genetic ablation of IFNAR or IRF3 (Stetson and others 2008), and Aicardi-Goutières syndrome is also associated with mutations in SAMHD1, a negative regulator of antiviral responses (Rice and others 2009).

Inflammasome assembly after Aim2/DNA interaction and production of IL-1 β and IL-18 may also contribute to systemic autoimmunity. In support of this possibility, IL-1 β levels are increased in most mouse lupus models, recombinant IL-1 β aggravated nephritis in NZB/W mice, and treatment with a soluble IL-1 receptor reduced disease in MRL-*Fas*^{lpr} mice (Schorlemmer and others 1993). Levels of IL-18 are also increased in sera of MRL-*Fas*^{lpr} mice, and injections of this cytokine accelerated kidney pathology, whereas induction of anti-IL-18 antibodies conferred protection (Bossu and others 2003). Interestingly, an allelic variant of another DNA-binding member of the HIN-200 family, p202 (encoded by *ifi202* in mice), was reported to be hyperexpressed in lymphoid cells and contribute to disease in NZB mice (Rozzo and others 2001). However, the mode by which p202 might promote disease in mice is unclear, as this molecule lacks a PYD domain and is, therefore, unlikely to induce an inflammasome and, in fact, p202 inhibited the formation of the Aim2-DNA inflammasome (Roberts and others 2009). Nonetheless, studies in mice have implied an interplay between p202 and Aim2, with deficiency in Aim2 causing induction of type I IFNs and p202, and p202 suppressing expression of the inhibitory Fc γ RIIB (Panchanathan and others 2010; Panchanathan and others 2011). Perhaps of relevance, upregulation of all 4 human HIN-200 homologs (*MNDA*, *IFIX*, *IFI16*, and *AIM2*) and a significant association with certain allelic variants of *IFIX* and *IFI16* have been reported in one ethnic group of patients with SLE (Kimkong and others 2009, 2010).

Indirect evidence also suggests the potential involvement of the RLR sensing system in the pathogenesis of autoimmune diseases. Thus, viral 5'-triphosphate RNA aggravated lupus nephritis in MRL-*Fas*^{lpr} mice (Allam and others 2008). Moreover, increased levels of RIG-I were detected in the epidermis of patients with psoriasis (Kitamura and others 2007; Prens and others 2008), synovial tissues from patients with rheumatoid arthritis (Imaizumi and others 2008), and epithelial cells lining the gut mucosa (Kawaguchi and others 2009). In addition, loss-of-function mutations in RIG-I, and more frequently in MDA5 (encoded by *IFIH1*), were associated with resistance to type I diabetes (Nejentsev and others 2009; Shigemoto and others 2009; Downes and others 2010), and mice lacking one copy of MDA5 developed transient hyperglycemia after infection with a β cell-tropic virus (McCartney and others 2011). These findings support a viral pathogenesis of type I diabetes, but self-nucleic acids derived from phagocytosed apoptotic materials may also contribute.

Apoptotic materials as triggers in lupus

Despite the evidence that self-nucleic acids, either free or complexed with IgG autoantibodies, promote systemic autoimmunity, there is a relative paucity of information regarding the exact composition of nuclear materials presented *in vivo* to the immune system. In fact, considering the broad specificity of autoantibodies in lupus, it has been proposed that nucleic acids and other associated self-antigens may be presented in the form of "particles" generated during apoptotic or necrotic cell death (Hardin 1986; Tan 1989; Casciola-Rosen and others 1994; Riemekasten and Hahn 2005; Munoz and others 2010). This possibility is supported by the finding that materials from apoptotic cells bind antinuclear and antinucleosomal antibodies *in vitro*, and such complexes are isolated from the plasma of patients with lupus (Ullal and others 2011). Other evidence of the inflammatory properties of apoptotic materials is the finding that dying cells taken up by cDCs trigger TLR-independent type I IFN production and enhance adaptive antigen-specific responses (Janssen and others 2006). Moreover, SLE is characterized by excessive production of apoptotic cells and/or defective clearance of apoptotic materials (Viorritto and others 2007). The potential contribution of defects in clearance of apoptotic materials is further supported by the development of lupus in humans and mice with C1q deficiency (Botto and others 1998; Lewis and Botto 2006), and in mice with genetic deletion of the peroxisome proliferator-activated receptor- δ , which decreases expression of C1q (Mukundan and others 2009). In addition, lupus-like autoimmunity develops in mice lacking serum amyloid P (Bickerstaff and others 1999; Paul and Carroll 1999), TAM receptor protein tyrosine kinases (Tyro3, Axl, and Mer) (Lu and Lemke 2001; Cohen and others 2002; Lemke and Rothlin 2008), or milk fat globule-epidermal growth factor 8 (MFG-E8), all molecules that contribute to the removal of apoptotic materials (Hanayama and others 2004). Further, injection of apoptotic cells or apoptosis-promoting agents enhanced autoantibody production and kidney disease, especially in lupus-predisposed mice (Mevorach and others 1998; Denny and others 2006), whereas disease was reduced when clearance of apoptotic cells was enhanced by opsonization with adiponectin (Takemura and others 2007). Finally, nuclear fragmentation of apoptotic cells by the endonuclease caspase-activated DNase (CAD) was required for induction of antichromatin and anti-snRNP autoantibodies in pristane-treated mice (Frisoni and others 2007).

Although the role of apoptotic materials as potential triggers of innate responses has primarily been addressed by using splenocytes or lymphocytes, other types of dying hematopoietic cells, such as neutrophils, may also release materials that can engage endolysosomal or cytosolic sensors for nucleic acids. Thus, the number of circulating apoptotic neutrophils in SLE positively correlated with disease activity (Courtney and others 1999), and a granulopoiesis gene expression signature was detected in PBMC of some patients with SLE (Bennett and others 2003). Recent studies have provided mechanistic evidence of how neutrophil-derived apoptotic materials are generated and may contribute to lupus pathogenesis (Garcia-Romo and others 2011; Lande and others 2011; Mantovani and others 2011). It was determined that mature neutrophils primed *in vivo* by type I IFNs die when exposed to anti-RNP autoantibodies, releasing web-like structures known as neutrophil-extracellular traps (NETs),

which contain DNA as well as the accessory factors LL37 and HMGB1. These NETs were detected in SLE sera and were shown to activate pDCs to produce high levels of type I IFN in a DNA- and TLR9-dependent manner. LL37-containing NETs were also reported to be associated with autoimmune small-vessel vasculitis (Kessenbrock and others 2009).

Microbial Triggers and Systemic Autoimmunity

Pathogens themselves or in combination with materials released from damaged tissues may constitute the trigger that initiates chronic inflammatory processes when responses are excessive or unchecked. Specifically, microbial infections are frequently associated with lupus disease flares, and Epstein-Barr virus (EBV) has been considered a major environmental risk factor for this disease (Poole and others 2006). Moreover, disease is enhanced in lupus-predisposed mice injected with bacterial or viral TLR ligands or mimics (Theofilopoulos and others 2005). Type I IFNs are the ultimate mediators of pathogenesis, as disease enhancement is not observed in IFNAR-deficient mice that are similarly injected (Braun and others 2003).

A clear example of requirement for microbial stimuli in the induction of systemic inflammatory diseases is that of mice carrying a hypomorphic mutation of the *Ptpn6* gene (Croker and others 2008). Mice homozygous for this mutation (termed *spin* for spontaneous inflammation) displayed inflammation and mononuclear cell infiltration in feet, salivary glands, and lungs, as well as antichromatin antibodies. The *Ptpn6* gene encodes the Src-homology-2-domain-containing protein tyrosine phosphatase SHP-1, which downregulates signaling from TCRs, BCRs, TLRs, integrins, and receptors for several cytokines and chemokines. The *spin* phenotype resembled, in an attenuated form, that of the viable *motheaten* (*me^v*) mice, which also carry hypomorphic *Ptpn6* mutations. Likewise, B cell-specific deficiency of SHP-1 promoted B-1a cell development and caused serologic and histologic manifestations of systemic autoimmunity (Pao and others 2007). With regard to *spin* mice, compound mutations of *Ptpn6* with MyD88, IRAK4, or IL-1R inhibited disease development, whereas those with STAT1 (affecting type I and II IFN signaling) or TNF- α had no effect, thus indicating that disease in this model is primarily driven by IL-1 β . The most striking finding was that disease was absent when *spin* mice were derived and bred in a germ-free environment, but reemerged when these mice were reconstituted in a pathogen-free environment. This indicates that superimposition of a microbial trigger, likely derived from the enteric flora, is required for disease expression (Croker and others 2008). The overall scenario for disease in this model is that an initial TLR stimulus induces expression of pro-IL-1 β , which, in a second step likely mediated by an inflammasome, is converted to mature, secreted IL-1 β . On binding its receptor, IL-1 β then creates an autoamplification loop that sustains the autoimmune/inflammatory process (Beutler 2009).

Conclusion

The interplay of genetic and environmental forces in the pathogenesis of diseases is a familiar concept, but the precise definition of these forces is often extremely difficult. This has certainly been the case for autoimmune diseases. The detailed characterization of the central pathways involved in

the initiation of innate immune responses has now provided us with some clearer hypotheses. A wide spectrum of sensors has been identified that are solely dedicated to the detection and containment of infection and cellular damage. However, the associated beneficial inflammatory responses may sometimes be excessive or uncontrolled, thereby contributing to the initiation of pathogenic autoimmunity. Microbes may be involved in this process and act as triggers or long-term drivers of pathologic responses in predisposed individuals. However, in some instances, microbes may be entirely excluded, and autoimmune responses may develop under "sterile" conditions as a true dysfunction of "self." However, the combined participation of both microbes and self-tissue-derived stimuli may also occur. In all these cases, the primary stimulus may be nucleic acids recognized by endosomal or cytosolic sensors, thereby leading to induction of type I IFNs and other proinflammatory cytokines. This concept may be applicable to a wide spectrum of autoimmune diseases, although it is most clearly documented for lupus. This mechanistic reformulation for the pathogenesis of autoimmune diseases will likely lead to new therapeutic avenues directed toward the inhibition of nucleic acid recognition, trafficking of sensors to specific cellular organelles where recognition occurs, downstream signaling events, and/or inhibition of end-stage mediators. It can be envisaged that interventions in these processes will soon find a place in modern medicine for the treatment of these frequently intractable disorders.

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Author Disclosure Statement

The authors declare that no competing financial interests exist.

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Address correspondence to:
Dr. Argyrios N. Theofilopoulos
Department of Immunology and Microbial Science
The Scripps Research Institute
La Jolla, CA 92137

E-mail: argyrio@scripps.edu

Dr. Roberto Baccala
Department of Immunology and Microbial Science
The Scripps Research Institute
La Jolla, CA 92137

E-mail: rbaccala@scripps.edu

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