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Spontaneous activation of RNA-sensing pathways in autoimmune disease

Steve P Crampton* and Silvia Bolland*

*Laboratory of Immunogenetics, National Institute of Allergic and Infectious Diseases, National Institutes of Health, Rockville, Maryland 20852

Abstract

Multiple intracellular RNA sensing innate immune pathways have been linked to autoimmune disease. RNA-related ligands taken up by the endocytic pathway activate TLRs, and affect primarily immune cells. This type of activation is enhanced by nucleic acid-specific antibodies and induces an inflammatory program. In contrast, spontaneous activation of cytoplasmic RNA sensing pathways target mostly non-hematopoietic tissues and their effect on autoimmune disease is secondary to the release of interferon in the circulation. The fact that pathologies result from spontaneous activation of innate pathways implies that endogenous RNA ligands that might be sensed as pathogenic are commonly found in both immune and non-immune cells.

Introduction

Evidence derived from genetic and immunological studies suggests that spontaneous activation, exaggerated duration or misregulation of innate viral sensing pathways can lead to autoimmune pathologies. Systemic autoimmunity frequently correlates with the presence of serum anti-nuclear antibodies and chronic expression of interferon inducible genes, symptoms proposed in association with nucleic acid sensing TLR-dependent activation of immune cells [1]. Other innate pathways that recognize viral replication intermediates in the cytoplasm have also been linked to both systemic and organ-specific autoimmune diseases, likely mediated by the effects of elevated type I interferon levels [2]. In this review, we reflect on differences between endocytic and cytosolic innate pathways and how that determines the cell types involved and consequent pathologies. We also discuss possible sources of nucleic acids, with emphasis on RNAs, which might induce spontaneous disease.

Viral nucleic acids activate endocytic and cytoplasmic innate pathways

Sensing of pathogen-derived RNA and DNA occurs in the cytoplasmic or endocytic compartments of cells, depending on the cell type and origin of the pathogen [3]. Endocytic

Corresponding author: Bolland, Silvia (sbolland@niaid.nih.gov), 12441 Parklawn Drive, Twinbrook 2, room 217, Rockville, MD, 20852.

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innate pathways comprise intracellular TLRs, which encounter foreign particles endocytosed by professional antigen-presenting cells such as B cells, macrophages or dendritic cells (Figure 1) [3]. TLR7 and TLR8 recognize ssRNA while TLR3 and TLR9 recognize dsRNA and unmethylated bacterial CpG DNA, respectively. Two pathways can be activated downstream of TLR7, TLR8 or TLR9: MyD88/NF κ B-dependent proinflammatory cytokine production and MyD88/IRF7-dependent IFN-I production [3]. TLR3 can activate similar pathways, except it uses TRIF as a proximal adaptor instead of MyD88 [3]. Induction of IFN-I (α/β) production seems to have differential requirements compared to those required for the generation of proinflammatory cytokines such as IL-6 or TNF α [4,5]. The type of endocytic pathway used to internalize the ligand, the specific cell lineage or cellular activation state might determine whether nucleic acid sensing promotes a heavy IFN-I response versus the more conventional inflammatory cellular program. In particular, IFN- α seems to be produced heavily by the more specialized plasmacytoid dendritic cells (pDC) while cells of the monocyte and B cell lineages respond preferentially by activating the NF κ B transcription program [6]. Generally, nucleic acid-sensing TLRs preferentially activate immune cells with MHC-II presentation potential, in a way that might expand immune recognition of self-antigen and lead to autoimmunity [7].

Cytoplasmic innate pathways detect the viral components within directly infected cells. The cytosolic RIG-I-like receptor family consists of RIG-I, MDA5 and LGP2. RIG-I recognizes 5'-triphosphorylated RNA while MDA5 recognizes dsRNA (Figure 1). Activation of these receptors leads to IFN-I production via the mitochondrial-associated adaptor IPS-1 and IRF3/IRF7-induced transcription [8]. Intermediates of viral replication are always sensed as a threat to the cell, especially when they are DNA-RNA hybrids or uncapped single stranded forms that the cell does not recognize as part of the replication or transcription machinery [9]. To avoid continuous activation of innate alerts, there are cell-encoded nucleases that very efficiently eliminate these hybrids, such as RNaseH2 and TREX [9]. As we will discuss below, any dysfunction in this protective system will lead to chronic activation of viral alert responses and will cause pathologies. In general, innate cytoplasmic pathways are ubiquitous and do not tend to activate inflammatory responses on their own, although IFN-I released into circulation might heighten immune responses as a secondary effect: circulating IFN-I can either enhance the antigen presentation ability of immune cells or promote self-epitope presentation by augmenting the rate of cell death and presence of cell debris [10].

Genes involved in RNA sensing during viral infections are linked to autoimmune disease

Both genome wide association studies (GWAS) in humans and genetic studies in mice have been invaluable in identifying genes associated with autoimmune diseases (Figure 2). They have revealed multiples links between nucleic acid sensing pathways and autoimmunity but also, importantly, they have identified qualitative differences among some pathways that had seemed analogous at first glance. For example, while multiple intracellular TLRs could all potentially be linked to autoantibody and IFN-I production, genetic studies uniquely place TLR7 as a susceptibility gene and exclude others from this classification. In mice, increased expression of *Tlr7* by two-fold accelerates autoimmunity in a lupus-prone mouse strain,

whereas higher expression drives a lupus-like autoimmune disease in an otherwise healthy mouse strain [11,12]. *Tlr7*-deficient mice are resistant to both induced and spontaneous forms of systemic lupus erythematosus (SLE) [13–15]. Additionally, and beyond the lupus pathology, TLR7 is required for maximal disease severity in experimental autoimmune myocarditis [16]. In contrast, increased dosage of *Tlr3* or *Tlr9* does not lead to spontaneous disease ([17], S. Crampton, T. Tarasenko and S. Bolland, unpublished data). Also, even though genetic ablation of *Tlr9* reduces autoantibodies to nucleosomes, disease is actually worsened in multiple mouse models of SLE [18]. The genetic information on TLR8 seems to be even more complex: *Tlr8*-deficiency in mice induces a lupus-like disease, seemingly mediated by increased TLR7 expression [19]. Mouse TLR8 appears nonfunctional by itself, contrasting to the ssRNA-responding human TLR8. Human and mouse TLR8 could also differ in ligand specificity in yet unknown ways. These findings might explain why expression of human TLR8 in mice results in a dose-dependent multi-organ autoimmune pathology (C. Guiducci et al., submitted). Thus, so far the evidence most clearly identifies TLR7, and possibly TLR8, as capable of spontaneous activation with pathological consequences. Human genetic studies seem to confirm this view, as single nucleotide polymorphisms (SNPs) associated with increased expression of *TLR7* were also identified in SLE patients from Eastern Asian countries [20,21].

Multiple signaling components downstream of intracellular TLRs have been shown associated with SLE: (1) IRAK1, which links endosomal TLRs with TRAF6 and downstream signaling, contains risk variants in SLE [22]. Lupus-prone *Sle1* and *Sle3* mice have reduced autoantibodies and disease when crossed to IRAK1^{-/-} mice [23] (2) Among the interferon response factors (IRFs) that target IFN-I gene transcription, *IRF5* and *IRF7* both contain SNPs associated with SLE [24]. *Irf5*-deficient mice show a diminished autoimmune response in lupus-prone *Fcγr2b*^{-/-} Yaa mice [25]. (3) *SLC15A4*, a cotransporter localized in the lysosomal membrane required for TLR-induction of IFN-I, is associated with both SLE and inflammatory bowel disease [26–28]. (4) Mutations in TNFAIP3, a negative regulator of NFκB signaling, shows association with several autoimmune diseases, including SLE, Rheumatoid Arthritis (RA), Crohn's Disease and Psoriasis [22]. This type of mutation thus results in a gain-of-function situation for the inflammatory pathway, equivalent to the chronic and spontaneous sensing described above in increased dosage of the receptor [11,19]. (5) Studies on the tyrosine phosphatase *PTPN22* have recently linked certain allelic forms with a defect in TLR-mediated IFN-I, but not inflammatory cytokine production [29]. This gene has been associated with a wide range of autoimmune disorders including SLE, RA and Myasthenia Gravis [30]. Although originally hypothesized to set activation thresholds for lymphocytes by dephosphorylating key proteins proximal to the antigen receptor, it is intriguing to think that the disease-associated SNPs might be primarily a gain-of-function mutations that affect innate responses [30]. Perhaps this effect of dysregulating the balance between inflammatory cytokines and IFN-I will prove to be the underlying cause of more genetic susceptibilities.

Genetic evidence has also linked various innate cytoplasmic sensors to autoimmune disease. SNPs in *IFIH1*, the gene that codes for MDA5, show a strong correlation with Type One Diabetes and SLE, perhaps associated with decreased expression or function of the protein

[31,32]. On the other hand, increased expression of MDA5 accelerates autoimmunity in the lupus prone *Fcγr2b*^{-/-} mouse [33]. Evidence from patients with the congenital autoimmune disease Aicardi-Goutières syndrome (AGS) demonstrates that clearance of unusual nucleic acid species in the cytoplasm of cells is critical for autoimmune suppression. Cohorts of these patients have mutations in the 3'-5' exonuclease *TREX1* and the ribonuclease *RNASEH2* [34,35]. Interestingly, *TREX1* mutations are also causal in a cutaneous form of SLE called familial chilblain lupus [36]. Consistent with this patient data, *Trex*-deficient mice also develop autoimmunity driven by IFN-I and originating in non-hematopoietic tissue [37,38].

How do RNA sensing pathways become pathogenic?

RNA sensing pathways are designed for the beneficial role of protecting against viruses and yet they are also linked to pathogenic autoimmune conditions. In fact, normal anti-viral immune responses commonly involve epitope spreading into cross-reactive and self-reactive specificities [39], which might even be beneficial to maintain long term immunological memory. In healthy individuals this response is transient and largely disappears once the pathogen is eliminated. In individuals susceptible to autoimmune pathologies, however, these responses become chronic, either because they happen spontaneously in the absence of infection or because they do not get resolved once the infection is eradicated.

Lymphocyte tolerance plays an important role in preventing disease, as the presence of antibodies with nucleic acid specificities facilitates spontaneous activation of endocytic TLRs. This concept was originally hypothesized by the group of Ann Marshak-Rothstein and amply verified since [40,41]. In B cells, the antibody forms an integral part of the B cell receptor (BCR), which binds and internalizes nucleic acids, allowing BCR and TLR to act in synergy to potentiate cell activation [42]. In monocytes and DCs, Fc receptors bind immune complexes carrying nucleic acids and internalize them to induce synergy between the FcR and TLR pathways (Figure 1) [43]. This model was first proposed to explain TLR9-dependent activation of B cells with dsDNA autoreactivity, important for the pathogenesis in SLE [40]. However, as we discussed above, genetic studies suggested a differential requirement for TLR9 versus TLR7 for disease pathogenesis in SLE, even though TLR7 and TLR9 are thought to activate similar downstream signaling components [3]. One theory is that TLR7 and TLR9 compete for an endosomal niche and RNA-containing ligands are more readily available than DNA ligands. In support of this theory, the exaggerated response against RNA-related antigens in *Tlr9*-deficient lupus-prone mice is eliminated when the mice also lack *Tlr7* [44]. Another theory is that TLR9 is important for inducing tolerogenic cell populations. Indeed, *Tlr9*-deficient mice displayed a defect in T regulatory cell activity [45]. On the molecular level, differential intracellular trafficking of TLR7 versus TLR9 could shed some light on the differences seen in disease [46]. The multi-pass transmembrane protein UNC93B1 is required for the function and endolysosomal targeting of all endosomal TLRs, including TLR7 and TLR9 [47,48]. A point mutation in *Unc93b1*, D34A, skews binding towards TLR7 and away from TLR9 [49]. Mice carrying this point mutation develop a TLR7-dependent lethal autoimmune disease reminiscent of TLR7 transgenic mice [11,50]. These data suggest that UNC93B1 may be limiting and normally favors binding to TLR9 over TLR7. In addition to UNC93B1-mediated trafficking, other factors have been

shown to differentially bind and sort TLR7 versus TLR9 to endosomes. Adaptor protein complex 4 (AP4) appears to direct TLR7 and AP2 directs TLR9 to endosomal compartments [51].

Another level of regulation of TLRs is cell type-specificity [6]. Not only do dendritic cell subsets express unique and non-overlapping TLRs, but they also can respond differentially to the same ligand, for example, pDCs produce IFN-I, while mDCs and B cells produce IL12 and proliferate after TLR7 stimulation, respectively [52]. This is relevant *in vivo*, as increased gene copy number of *Tlr7* preferentially expands germinal center B cells and plasmablasts over pDCs [53]. Thus, intracellular TLRs involved in autoimmune disease seem to especially target immune cells that directly alter antibody levels and IFN-I. This is a consequence of the need for ligand uptake through endocytosis plus the relatively narrow spread of expression of the TLRs. In contrast, spontaneous activation of cytoplasmic innate pathways seems to target non-hematopoietic tissues, perhaps triggered by infrequent cell damage activation events that initiate a larger cascade of inflammatory responses. These infrequent events probably occur in healthy individuals but are likely amplified by genetic modifications that might enhance the response or increase tissue damage. Both *Trex*-deficient mice and mice with increased dosage of MDA5 show spontaneous release of IFN-I that is not bone marrow-derived, yet circulating IFN-I ultimately alters the immune cells activation state and homeostasis with consequences in autoimmune susceptibility [33,37]. Most importantly, it seems that pathways that sense and eliminate non-mammalian RNA and other unusual nucleic acid forms produced through viral replication need to be tightly regulated because their endogenous ligands are likely to appear even in the absence of infection and they have the potential to incite autoreactivity. Perhaps that is the reason why expression of RNA sensors and related genes is commonly induced by interferon so that the potential for pathogenicity is reduced in the absence of viral infection.

RNAs involved in autoimmunity: mammalian or virally derived?

There is convincing evidence that inappropriate RNA sensing leads to autoimmune pathologies, but the source of the RNA ligands that promote this immune dysfunction is uncertain. It is possible that some genetic modifications result in ligand-independent activation of the pathway, resulting in a chronic and spontaneous signal, although even this situation could be subject to environmental regulation. One case that might be explained this way is the pathology induced by increased dosage of the *Tlr7* gene [11,12]: spontaneous disease could be explained by the random formation of TLR7 receptor dimers that would not require an RNA ligand for signal transduction. Against this possibility is the finding that transgenic expression of RNase reduces spontaneous pathology induced by increased activity of TLR7 in these mice [54].

If spontaneous TLR7 activation requires the presence of endogenous RNA to activate the pathway, this could originate from the nuclear remains or extruded vesicles of apoptotic or necrotic cells, which are normally taken up and disposed of by macrophages [55]. They could also come from circulating RNA containing nuclear particles captured as immune complexes, which have been found to stimulate the production of IFN-I by pDCs [56]. There is evidence that mammalian RNA contains structural modifications preventing the

activation of TLR7 [57]. These modifications, however, alone are not enough to prevent activation. Mammalian RNAs are variable in their levels of modification, an even when non-immunogenic by themselves they can stimulate TLR7- and TLR3-dependent proliferation and cytokine secretion of B cells if delivered using immune complexes [58,59].

RNA derived from endogenous retroviruses (ERVs) is another possible source of stimulation for RNA sensors in autoimmunity. A recent study demonstrated that TLR7 and IRF5 controls reemergence of ERV viremia in mice, suggesting that these RNA viruses under basal conditions are actively engaging TLR7 [60]. Another intriguing possible source of stimulatory endogenous RNA is small genomically-encoded microRNA (miRNA). Recently, it was demonstrated that let-7b, a miRNA abundant in the CNS, was capable of causing neurodegeneration by stimulating the TLR7/MYD88 pathway in neurons [61]. Let-7b was elevated in the cerebrospinal fluid of Alzheimer's patients, most likely as a result of neuronal cell death. This raises the possibility that miRNAs released from apoptotic or necrotic cells could promote TLR7-dependent autoreactivity.

Cytosolic RNA and DNA sensors can be activated by viral replication intermediates in the absence of an active infection, perhaps by reactivated endogenous retroviruses. UV light could be an environmental trigger in this case as it can induce damage of nucleic acids or even the reactivation of retroviruses. UV damage of nucleic acids has been hypothesized to be the underlying cause of skin sensitivity in many autoimmune diseases [62]. The fact that patients with AGS, linked to mutations in protective nucleases, develop a severe pathology early in life [34,35], argues that appearance of nucleic acids sensed by the cytoplasmic innate system is a common occurrence in spontaneous conditions. *Trex* deficiency in mice seems to originate in tissues like the heart that endure the most physical stress, as if localized cell damage or release of nuclear components might incite IFN-I production [37,38]. Evidence suggests that by increasing cellular RNase L activity, RNase L-processed endogenous self or viral RNA spontaneously stimulates the production of IFN-I [63]. These RNase L-processed cellular RNAs are able to activate IFN- β transcription through MDA5 and RIG-I. It is interesting that innate cytoplasmic activation usually induces IFN- β as the major cytokine, which might have differential effects, perhaps more regulatory, than the many IFN alpha species [64,65]. Indeed, recently, an IFN- β -specific transcription program was revealed [66]. With this difference in mind, future clinical studies might have to qualify whether an increased in interferon signature expression is due to alpha or beta IFN-I production.

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* of special interest

** of outstanding interest

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Highlights

- RNA from viral replication intermediates stimulate endosomal/cytoplasmic sensors.
- Autoimmunity develops from mutations in protective cytoplasmic nucleases.
- Genetic studies indicate RNA sensing pathways in autoimmunity.
- Pathogenic stimulatory RNA may come from self or viral sources.

Figure 1.

Ways in which RNA can activate innate pathways. (a) Endocytic innate pathways. (left) Extracellular RNA can enter B cells through binding to the B cell receptor, endocytosis and fusion with a TLR7-containing endolysosome. Acidification of the lysosome then facilitates binding of RNA with TLR7 and subsequent downstream activation of NF κ B-dependent proliferation and proinflammatory cytokine production. (right) RNA-containing immune complexes can enter plasmacytoid dendritic cells through Fc receptors. Subsequent activation of TLR7 can then activate high levels type one IFN. (b) Cytoplasmic innate pathways. RNA or DNA derived from viral infection of non-lymphoid tissues can activate cytosolic innate pathways leading to IRF3-dependent IFN-I production and subsequent Interferon stimulated gene (ISG) expression. Cytosolic 5'-triphosphorylated ssRNA or dsRNA derived from viral replication intermediates can activate RIG-I and MDA5, respectively. UV-damaged nucleic acid products can also activate cytosolic pathways.

Figure 2.

Genetic hits of innate pathways in autoimmunity. Human and/or mouse studies have identified susceptibility genes in autoimmunity (in red). Many of these genes code for proteins involved in nucleic acid recognition in both endocytic and cytoplasmic compartments. SLC15A4, a proton-coupled Histidine and oligopeptide cotransporter localized in the lysosomal membrane is involved in endocytic recognition of TLR ligands. IRAK1, IRF5/7 and PTPN22 are all involved in TLR-dependent activation of IFN-I. TNFAIP3 is a negative regulator of NFκB responses. MDA5 recognizes cytoplasmic dsRNA and activates IRF3/7 through Mitochondria-associated IPS-1. TREX1 and RNASEH2 are nucleases that digest excess nucleic acids, for example, coming from viral replication intermediates.