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Connections between antiviral defense and autoimmunity

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Summary

Recent advances have revealed a fundamental contradiction in antiviral immunity: innate immune sensors that detect nucleic acids mediate both protective immunity to infection and pathological autoimmune disease. Thus, study of the mechanics of nucleic acid detection will provide insight into how these systems are inappropriately triggered in autoimmunity, and, conversely, study of autoimmune disease triggered by these sensors will tell us more about how they are linked to activation of adaptive immunity.

Introduction

Nucleic acid parasitism is perhaps the most ancient form of infection. All organisms have viral pathogens, and billions of years of host-virus evolution have produced a number of fascinating mechanisms of antiviral defense. These antiviral systems vary among phyla, from restriction enzymes and the CRISPR-mediated anti-phage defense in prokaryotes [1], to RNA interference in plants, worms, and flies [2], and finally to the diverse and elaborate antiviral mechanisms that exist in vertebrates [3,4]. Importantly, all of these systems are based on the detection of nucleic acids, thus raising a fundamental question of self/non-self discrimination: antiviral sensors must detect foreign genetic material among the abundance of host-derived DNA and RNA. Recent insights suggest two imperfect evolutionary solutions to this problem of self/non-self discrimination: antiviral sensors detect (a) modifications or structural features of foreign nucleic acids that are generally not present in the host, or (b) the unscheduled appearance of nucleic acids in the wrong cellular compartment. These rules of discrimination are not absolute, and emerging evidence implicates the same antiviral systems that protect us from infection as the cause of numerous autoimmune syndromes. Importantly, the underlying mechanisms that mediate protective versus autoreactive antiviral responses are not just similar, but they are likely to be identical. During viral infection, the antigens are foreign and the response is protective. In autoimmunity, the antigens are endogenous and the response is destructive.

In this review, I will summarize the mounting evidence that implicates aberrant nucleic acid detection as the basis of numerous autoimmune disorders and attempt to integrate this understanding into a broader model of autoimmunity. I will then consider potential sources of endogenous nucleic acids that may trigger tissue-specific autoimmunity. Finally, I will focus on a fundamental, unanswered question: how is the innate antiviral response linked to the activation and function of autoreactive lymphocytes that mediate autoimmune pathology?

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Insight into this important question will provide a foundation for new treatments of autoimmune disorders and will reveal a clearer path towards the rational design of better vaccines.

Mechanisms of nucleic acid detection

In vertebrates, the innate antiviral response triggered by nucleic acid sensors is tightly linked to activation of adaptive immunity through the induction of the type I interferon (IFN) family of cytokines. The activation of IFNs and their varied roles in host defense and autoimmunity have been extensively reviewed elsewhere [5–7], and a number of general principles have emerged. The vertebrate nucleic acid sensors that activate the IFN response can be placed into two classes based on the cell types that express them, the subcellular location of the sensors, and the source of the nucleic acids that trigger the antiviral response. One class consists of several Toll-like receptors, which are expressed by key sentinel cells of the innate immune system, including dendritic cells and macrophages [8]. TLRs that sense nucleic acids are located in endosomal compartments and sample phagocytosed material for the presence of viral nucleic acids. Thus, the TLR system comprises a non-cell autonomous system in that the source of viral nucleic acids is distinct from the responding cells. During infection, many viruses encode proteins that directly antagonize IFN induction and response within infected cells. Importantly, because the TLR system is non-cell autonomous, it is generally refractory to such inhibition.

A second class of receptors detects nucleic acids within the cytosol of the infected cell itself and mediates a cell-intrinsic, IFN-mediated antiviral response. For such a system to be effective, it must be broadly expressed, especially in non-hematopoietic cells that are common portals of viral entry like epithelia. This cell-intrinsic system is comprised of sensors of viral RNA and DNA, among which the RNA helicases RIG-I and MDA5 are the best characterized [9]. Cytosolic sensors of viral DNA include DAI and other, currently unknown receptors [10–12]. Importantly, these sensors and the signaling pathways that they activate are the key targets of viral antagonism within infected cells.

Together, cytosolic receptors and TLRs activate non-redundant and complementary mechanisms of antiviral defense. Indeed, recent studies have revealed that mice lacking either or both of these antiviral pathways are highly susceptible to viral infection and fail to develop protective immunity after vaccination [13–15]. Moreover, emerging evidence implicates both TLRs and cytosolic sensors as the cause of numerous severe autoimmune disorders, thus revealing a key intersection between antiviral defense and autoimmunity with implications for how we classify and treat autoimmune disease.

Nucleic acids, interferons, and autoimmunity

In recent years, evidence has emerged implicating dysregulated type I IFN production as a key pathological feature of numerous autoimmune diseases [7,16]. In parallel, the sensors that activate type I IFNs were discovered and shown to detect nucleic acids [9,17,18]. Thus, the source of type I IFNs that trigger autoimmunity must be tied to these nucleic acid sensors. Importantly, just as there are two classes of nucleic acid sensors, the nucleic acids that trigger autoimmunity can be similarly grouped depending on which class of sensor they activate.

There is extensive evidence for an important contribution of TLR-mediated detection of endogenous nucleic acids to autoimmunity. First, general deficits in nucleic acid metabolism or clearance can be sensed through TLRs. For example, loss of function mutations in DNase I, which metabolizes extracellular DNA, are associated with lupus-like diseases in mice and humans [19,20]. Second, protection of extracellular nucleic acids from degradation can facilitate delivery to the endosomal compartments from which nucleic acid-sensing TLRs

signal, leading to pathological consequences. An interesting example of this was recently found in human psoriasis, where a complex of DNA and the antimicrobial peptide LL37 was found to activate TLR9-dependent IFN production by plasmacytoid dendritic cells (pDCs) [21]. Finally, heightened sensitivity of TLR signaling can lower the threshold for triggering by self-derived nucleic acids: a spontaneous gene duplication of murine TLR7 predisposes to autoimmunity [22–25].

One important mechanism by which extracellular nucleic acids cause autoimmunity is through activation of TLRs on autoreactive B cells [26]. TLR7 and TLR9 are important for the generation of autoantibodies to RNA- and DNA-protein complexes, respectively, in human lupus and in the MRL-*lpr/lpr* murine lupus model [27,28]. Interestingly, loss of TLR7 ameliorates disease while TLR9 deficiency exacerbates pathology in mice [28], suggesting a complex response to different TLR-dependent autoantigens, with some being more pathological than others.

In addition to TLR-dependent autoimmunity, recent studies suggest that cell-intrinsic antiviral sensors can also drive severe autoimmune disease. Nagata and colleagues found that liver macrophages in mice lacking the lysosomal DNase II become engorged with the nuclei of erythrocyte precursors and develop a TLR-independent IFN response to this undigested DNA that results in lethal anemia [29,30]. Moreover, type I IFN expression by pancreatic beta cells was observed in type I diabetes patients over two decades ago [31], and IFNs contribute to disease in murine diabetes models [32]. Intriguingly, polymorphisms in MDA5 were recently identified in genome-wide association studies of human type I diabetes, suggesting a possible mechanistic explanation that will require further study [33–35]. Finally, it was recently shown that detection of cytosolic DNA can result in activation of the inflammasome, a caspase-1 activating complex that processes interleukin 1 and other inflammatory cytokines [36]. Interestingly, AIM2 was recently identified as the DNA sensor of this pathway [37–40]; and a key negative regulator of the AIM2 inflammasome is a lupus susceptibility allele in mice [40,41], suggesting an interesting new connection between inflammasome activation and autoimmune disease.

One specific human autoimmune disease has provided important insight into connections between cell-intrinsic nucleic acid detection and severe autoimmunity. Aicardi-Goutieres Syndrome (AGS) presents in infancy as a severe encephalitis with lymphocyte infiltrates in cerebrospinal fluid, elevated type I IFN levels, and demyelination of motor neurons [42,43]. Many of these symptoms resemble those caused by congenitally acquired viral infection [44]. Remarkably, however, no viral pathogen has ever been detected in AGS patients.

In 2006, Yanick Crow and colleagues identified loss of function mutations in the gene encoding 3' repair exonuclease 1 (Trex1) in AGS patients [45]. Trex1 enzymatic activity was originally described 40 years ago as the most abundant 3'→5' DNA exonuclease in mammalian cells [46], and the Trex1 gene was cloned 30 years later [47,48]. Interestingly, Trex1 protein is extranuclear and is localized to the cytosolic face of the endoplasmic reticulum [49]. Since the groundbreaking report of Trex1 mutations in AGS, more than thirty independent mutations in Trex1 have been identified. While most of these mutations are recessive alleles that cause AGS, several Trex1 mutations cause or are associated with different diseases. First, dominant mutations in the catalytic domains of Trex1 cause familial chilblain lupus (FCL), a cutaneous form of systemic lupus erythematosus characterized by lesions at the extremities of fingers, toes, and ears [50,51]. Second, Trex1 mutations are found at higher frequency in SLE patients than in healthy controls [52]. Finally, heterozygous mutations resulting in a truncated, mislocalized, but enzymatically active form of Trex1 cause retinal vasculopathy with cerebral leukodystrophy (RVCL), a disease characterized by abrupt onset of visual loss, stroke, and dementia in middle age followed by death within 5–10 years [53]. RVCL bears little phenotypic

resemblance to AGS, suggesting that these distinct diseases are caused by loss of function versus unnatural gain of function in Trex1, respectively.

Trex1-deficient mice develop inflammatory myocarditis and suffer premature mortality [54]. While the symptoms of disease differ between humans and mice, these mice remain an excellent model to explore the molecular mechanisms that underlie AGS. Interestingly, Trex1-deficient cells accumulate extranuclear DNA [55], and this DNA chronically triggers cytosolic DNA sensors to initiate lethal, IFN-dependent autoimmunity [56]. Thus, AGS represents a specific example of autoimmunity initiated by cell-intrinsic nucleic acid detection, and reveals a mechanism that can be incorporated into current models of autoimmune disorders.

A continuum model of autoimmunity

The roots of autoimmune disease – both genetic and environmental – are diverse and complex, but the emerging evidence described above hints at a number of basic principles that may offer insight into how to classify such diverse disorders. There are at least three paths to the accumulation and activation of autoreactive lymphocytes, each of which has a distinct mechanistic basis (Figure 1). First, specific defects in central or peripheral lymphocyte tolerance allow accumulation of self-reactive lymphocytes that would normally be deleted or anergized (Figure 1; examples of specific genes are shown in blue). Such defects broadly impact the repertoire and sensitivity of antigen receptors and generally lead to multi-organ or systemic autoimmune diseases. Second, dysregulated activation of non-cell autonomous antiviral responses through TLRs can lead to IFN-dependent pathology through effects on dendritic cells and direct co-activation of TLRs and antigen receptors on B cells (Figure 1, green boxes). Third, cell-intrinsic initiation involves the chronic activation of cytosolic nucleic acid sensors by intracellular nucleic acids (Figure 1, red boxes). While the precise mechanisms that connect cell-intrinsic initiation to activation of adaptive immunity remain to be established, it is important to note that these mechanisms are probably identical to those that link cell-intrinsic detection of viral infection to protective immunity.

There are a number of implications that arise from a continuum model of autoimmune disease. First, tolerance deficits, because they directly affect the size and repertoire of the pool of circulating autoreactive lymphocytes, may not require an innate immune stimulus to drive autoimmunity. For example, TLRs are not required for systemic autoimmunity caused by Aire deficiency or regulatory T cell dysfunction [57,58]. Instead, lymphocytes that escape tolerance may be driven by selective pressures like clonal competition for niches and survival signals [59]. Second, just as defective tolerance may not require an innate immune trigger, the converse may also be true for cell-intrinsic initiation: chronic activation of cytosolic nucleic acid sensors may drive autoimmunity without a requirement for defective tolerance. AGS is a particularly illustrative example: AGS cases with highly similar presentation have been documented worldwide among several ethnic backgrounds, with presumably diverse MHC alleles and lymphocyte repertoires in affected individuals [60]. Similarly, Trex1-deficient mice develop a highly stereotyped disease in the context of a normally selected compartment of lymphocytes on a genetic background that is not predisposed to autoimmunity [54,56]. Importantly, cell-intrinsic initiation places the focus on the initiating cells, not the lymphocytes, and thus may help explain the origins of tissue-specific autoimmunity. Major unanswered questions include the source of the endogenous nucleic acid triggers and the nature of the tissue-specific autoantigens that drive disease.

Endogenous nucleic acid triggers of autoimmunity

The nucleic acids that accumulate and chronically activate antiviral sensors are the key triggers of numerous autoimmune disorders. In the case of TLR-dependent autoimmunity to extracellular nucleic acids, the protein-nucleic acid complexes that drive autoreactive antiviral

immunity have been extensively characterized, as discussed elsewhere [61]. In contrast, nucleic acids that drive cell-intrinsic initiation remain enigmatic. Such intracellular nucleic acids could be derived from infectious viruses in some settings, with molecular mimicry driving autoreactive lymphocyte responses to self antigens [62]. However, there are potential sources of endogenous (non-infectious) nucleic acids that may trigger an antiviral response if they are not properly metabolized. In AGS, the identification of specific causative gene mutations provided key insights and established a foundation for future exploration of these endogenous nucleic acids. Crow and colleagues found that in addition to Trex1, mutations in the human RNaseH2 enzyme also cause AGS [63], suggesting that accumulation of RNA-DNA hybrids might drive disease. Such RNA-DNA hybrids could arise from at least three cellular processes: lagging strand synthesis of Okazaki fragments during DNA replication (which requires an RNA primer) [55], hybridization of nascent RNA transcripts to the template DNA strand, or reverse transcription of cellular RNAs and endogenous retroelements. Interestingly, DNA fragments derived from endogenous retroelements were abundantly recovered from Trex1-deficient cells, and Trex1 can metabolize reverse-transcribed DNA [56]. Thus, reverse-transcription may generate immunostimulatory nucleic acids, which would normally be metabolized by the concerted activities of RNaseH2 and Trex1. However, much more work is required to thoroughly characterize the nucleic acids that accumulate in AGS cells. A more complete understanding of their origin will undoubtedly lead to better strategies to prevent their appearance or persistence, and will provide important insight into the cause of AGS and related diseases.

Origin of autoantigens in antiviral autoimmunity

Because autoreactive lymphocytes drive autoimmune pathology, the source of the targeted autoantigens ultimately mediates the tissue specificity of disease. As discussed above, the continuum model reveals distinct underlying mechanisms that drive the effector function of self-reactive lymphocytes. These distinct mechanisms determine the nature of the targeted autoantigens and suggest a predictive model for their origin. Autoantigens targeted in the context of defective tolerance can be diverse and derived from multiple tissues, determined by the types and relative abundance of lymphocyte clones that escape negative selection or anergy. In contrast, autoantigens in TLR-mediated autoimmunity are invariably complexes of nucleic acids and proteins, which allows prediction of TLR contributions to other autoimmune disorders. Less clear, however, is the origin of autoantigens during cell-intrinsic initiation of autoimmunity. For example, Trex1-deficient mice develop abundant autoantibodies to a relatively restricted number of highly expressed heart tissue autoantigens, but these autoantibodies do not appear to target chromatin or ribonucleoproteins [56]. This raises the question of how dominant autoantigens are selected during cell-intrinsic initiation. One possibility is that proteins within the initiating cell are targeted through a process that is normally intended to facilitate presentation of viral antigens during infection, but becomes mistargeted in autoimmunity because the initiating cells detect nucleic acids in the absence of viral proteins. This process may involve transfer of both nucleic acids and targeted proteins upon phagocytosis of an apoptotic initiating cell by a professional APC (Figure 1). The dominant autoantigens would therefore be determined within the cell types that accumulate endogenous nucleic acids. Moreover, these dominant autoantigens would invariably be abundant proteins within the initiating cell, reflecting the rare mistargeting of an otherwise tightly regulated process. In other words, if this process could lead to presentation of any endogenous antigen and rarely “misfires”, chances are that an abundant protein would be the target of that rare misfire. Indeed, the abundant expression levels of dominant autoantigens in many tissue-specific autoimmune disorders is puzzling, because the most abundant endogenous proteins should drive the most complete peripheral lymphocyte tolerance. It therefore seems that this process is capable of overcoming tolerance to even the most abundant self-antigens. Thus, study of how dominant autoantigens are selected in the context of cell-

intrinsic initiation will be instructive not only for the design of better therapies for autoimmunity, but also for efforts to harness such powerful mechanisms to improve the immune response to vaccines.

Conclusions and future perspectives

The recent advances described above reveal an inextricable connection between antiviral responses and many autoimmune diseases. Considering the long evolutionary history of the host-virus relationship, it seems that inherent autoreactivity of nucleic acid sensors is a necessary tradeoff to afford maximal protection against infection. Importantly, just as the protective and potentially harmful consequences of nucleic acid detection are intertwined, research into the logic of this innate immune strategy will provide important insight into both areas.

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Figure 1. A continuum model of autoimmunity

At least three mechanisms can drive activation of lymphocyte-dependent autoimmune pathology, each with unique requirements for regulation and distinct connections to adaptive immunity. Examples of each mechanism and the points at which they affect autoimmunity are shown in the highlighted boxes: (a) defective lymphocyte tolerance (blue boxes); (b) dysregulated TLR detection (green boxes); and (c) cell-intrinsic initiation by cytosolic nucleic acid sensors (red boxes). Please see the text for details.