REVIEW

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The enemy within: endogenous retroelements and autoimmune disease

Hannah E Volkman & Daniel B Stetson

Inappropriate or chronic detection of self nucleic acids by the innate immune system underlies many human autoimmune diseases. We discuss here an unexpected source of endogenous immunostimulatory nucleic acids: the reverse-transcribed cDNA of endogenous retroelements. The interplay between innate immune sensing and clearance of retroelement cDNA has important implications for the understanding of immune responses to infectious retroviruses such as human immunodeficiency virus (HIV). Furthermore, the detection of cDNA by the innate immune system reveals an evolutionary tradeoff: selection for a vigorous, sensitive response to infectious retroviruses may predispose the inappropriate detection of endogenous retroelements. We propose that this tradeoff has placed unique constraints on the sensitivity of the DNA-activated antiviral response, with implications for the interactions of DNA viruses and retroviruses with their hosts. Finally, we discuss how better understanding of the intersection of retroelement biology and innate immunity can guide the way to novel therapies for specific autoimmune diseases.

The sensing of nucleic acids by the innate immune system has emerged as the predominant means by which host cells detect the presence of viral infection[1]. Since 2004, two key intracellular sensing pathways have been discovered that mediate the detection of either RNA or DNA by the innate immune system. The sensors of RNA are the RIG-I-like receptors (RLRs) RIG-I and Mda5 (ref. 2); these detect structural features of viral RNA that are distinct from those of host RNA, and they activate a potent antiviral response that includes the inducible production of the type I interferon family of cytokines[1]. Activation of the RLRs results in their binding to MAVS, a transmembrane protein on the surface of mitochondria that serves as an adaptor between the sensors and the kinase TBK1 and transcription factor IRF3 that activates the production of type I interferons[1]. In contrast, the receptors for intracellular DNA (discussed in detail below) activate STING, a transmembrane protein on the endoplasmic reticulum that fulfills an adaptor function similar to that described above to link the detection of DNA to the TBK1-IRF3–dependent antiviral response[1,3] (**Fig. 1**). In addition to its role as an adaptor in the detection of DNA, STING is also directly activated by cyclic dinucleotides produced as second messengers by many species of bacteria[4]. Finally, the detection of intracellular DNA by the receptor AIM2 activates the ASC inflammasome[5–8], a signaling platform for production of the cytokines interleukin 1β (IL-1β) and IL-18 and a proinflammatory form of cell death called 'pyroptosis'[1]. Various published reviews have outlined the details of the sensing of intracellular RNA and DNA[9,10] that are only summarized here.

Department of Immunology, University of Washington School of Medicine, Seattle, Washington, USA. Correspondence should be addressed to D.B.S. (stetson@uw.edu).

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These pathways for the sensing of intracellular nucleic acids are crucial for signaling the presence of viruses within infected cells; thus, they are essential for the activation of a productive antiviral response. However, the same sensors of nucleic acids that provide protection from viral infection are also responsible for various human autoimmune diseases. Perhaps the most well-studied example of that is the involvement of the nucleic acid–sensing Toll-like receptors in the pathological autoimmune response to complexes of self nucleic acids and proteins[11–13]. Another source of endogenous immunostimulatory nucleic acids is a class of viruses present in the human genome: endogenous retroelements. Indeed, sensing of the reverse-transcribed DNA (cDNA) intermediates of such viruses by the innate immune system does occur, and exciting studies have shown that this mechanism of detection is also relevant for immune responses to human immunodeficiency virus (HIV).

In this Review, we first describe the dynamics of endogenous retroelements in cells. We then highlight the discovery of enzymes that metabolize endogenous retroelement cDNA and how key insights into the genetics of a rare human autoimmune disease have elucidated a new area of biology. We summarize fascinating advances in the understanding of responses of the innate immune system to infectious retroviruses. Then we discuss the implications of the sensing of cDNA for the evolution of DNA-activated antiviral responses. Finally, we propose that interventions that prevent the formation of retroelement cDNA may hold promise for the treatment of various human autoimmune disorders.

The life and times of endogenous retroelements

Retrotransposons replicate through a 'copy-and-paste' mechanism, inserting new copies of themselves into unique genomic locations. They have undergone several episodes of substantial expansion in number in mammals and constitute over 40% of the base content of the human genome[14–16]. Two families of retrotransposons are autonomous in that they encode all the proteins required for the reverse transcription of an RNA intermediate into a double-stranded DNA product that can be

inserted into a new place in the genome. The retrovirus-like long terminal repeat (LTR) retrotransposons are relics of ancient retroviruses that once integrated into the germline but then lost their ability to infect other cells. Their genomic organization and replication cycle resembles that of extant infectious retroviruses such as HIV, with reverse transcription of the viral mRNA primed by a specific cellular tRNA (**Figs. 1** and **2**). LTR retrotransposons make up about 8% of the human genome and are a stable population that is transcriptionally active but does not expand through new integration. However, those human endogenous retroviruses continue to influence genome organization, in large part through the high rate of recombination between their LTRs[17]. Retrotransposons of the 'long interspersed nuclear element 1' (LINE-1, or L1) family are the only actively replicating retroelements in the human genome. L1 retrotransposons are about 6 kilobases in length and encode two proteins, ORF1 and ORF2, that coordinate replication through target-primed reverse transcription; this involves the generation of a 'nick' in genomic DNA by the endonuclease activity of ORF2, followed by ORF2-dependent reverse transcription of L1 mRNA into cDNA at the precise site of the intended retrotransposition (**Figs. 1** and **2**). Thus, the LTR retroviruses and the L1 elements undergo reverse transcription at distinct cellular sites, with the former occurring in a capsid-enclosed virus-like particle in the cytosol and the latter occurring in the nucleus. A third family of retrotransposons called 'short interspersed nuclear elements' (SINEs) are nonautonomous and do not encode any proteins (**Fig. 1**). Most human SINEs are of a single type known as 'Alu', which at 300 base pairs are much smaller than the L1 retrotransposons[15,16]. However, Alu elements are very successful at replicating themselves, and they 'hijack' the L1 reverse transcriptase to mediate their retrotransposition in *trans*. There are over 1×10^6 copies of Alu in the human genome, which means there is one Alu for every ~3×10^3 base pairs of genomic sequence[14–16]. In addition to reverse-transcribing Alu RNA, the L1 reverse transcriptase can act on any polyadenylated mRNA to generate a processed pseudogene, which is an insertion of an intronless, promoterless cDNA into a unique genomic location. According to the latest estimates[18], there are 145 full-length, functional L1 elements in the human genome, together with 103 additional L1 elements with an intact ORF2 but a mutant ORF1. Those 'ORF2-only' L1 elements may be functionally relevant; whereas both ORF1 and ORF2 are required for the retrotransposition of L1 RNA, ORF2 can mediate the retrotransposition of Alu elements independently of ORF1 (ref. 19). With 248 known copies of functional L1 reverse transcriptase, the human genome therefore has the ability to create an enormous amount of retroelement cDNA. Indeed, recent studies have found that L1 insertions into the human germline occur at a rate that vastly exceeds prior estimates[20–23]. Given the difficulty of identifying insertions of new, polymorphic retroelements among the millions that already exist, such studies have probably underestimated the actual rate at which retroelements are inserted. Moreover, insertion of retroelements clearly occurs in somatic cells[24], perhaps at a rate even higher than that detected in the germline. Collectively, these studies have revealed the staggeringly large amount of ongoing diversification of the human genome created by mobile genetic elements. Various excellent reviews have summarized the biology and fascinating properties of such endogenous retroelements[15,16,25]; we will focus here on their potential to trigger the innate immune system and cause specific autoimmune diseases.

The intersection of retroelements and innate immunity

The sensing of retroelement cDNA by the innate immune system was fortuitously discovered during a search for candidate sensors of the antiviral response to intracellular DNA called the 'interferon-stimulatory DNA' (ISD) pathway[26,27]. Proteins that bound to transfected ISD were recovered with the hope of finding the sensor of ISD, and the first protein identified by mass spectrometry was the 3′ repair exonuclease Trex1, whose activity was first described over 40 years ago[28]. The study of Trex1 and its relation to the ISD pathway was crucially influenced by the discovery of mutations in the human *TREX1* gene that result in Aicardi-Goutières syndrome (AGS), a rare and severe autoimmune disease[29]. AGS is an early-onset, type I interfeon–associated disorder characterized by neurological dysfunction, psychomotor retardation and skin inflammation[30,31]. The finding of *TREX1* mutations in AGS and the subsequent identification of all other known AGS-related genes[32–34] established the framework in which the functions of the enzymes encoded by those genes are still interpreted. In addition to their association with AGS, *TREX1* mutations have also been identified in familial chilblain lupus, a monogenic form of cutaneous lupus[35,36]. Finally, heterozygous mutations in *TREX1* have been identified in a small subset of patients with systemic lupus erythematosus (SLE)[37,38] but are very rare in healthy control subjects. In fact, the association of *TREX1* mutations with SLE remains the strongest single association of a gene with this autoimmune disorder identified so far (according to the odds ratio)[39].

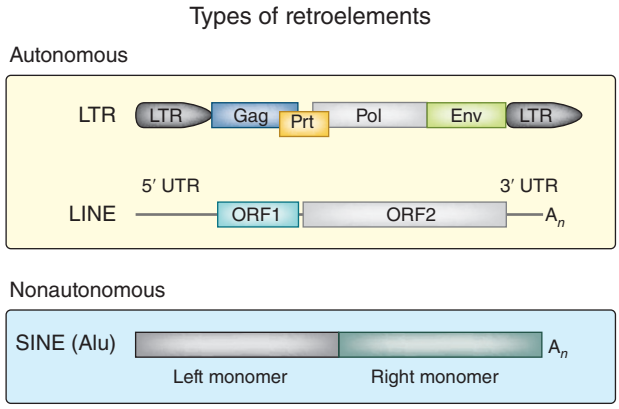

Figure 1 Types of endogenous retroelements in the mammalian genome. Autonomous retroelements (top) encode all the proteins necessary for replication. These include LTR-type endogenous retroviruses and LINEs. Nonautonomous retroelements (bottom) include SINEs and require LINEs for their mobility. Gag, group-specific antigen; Prt, protease; Pol, polymerase; Env, envelope; UTR, untranslated region; A_n, poly(A) tail.

Using Trex1-deficient mice as a model of AGS disease mechanisms, we found that the lethal autoimmune disease in these mice requires STING, IRF3, type I interferons and lymphocytes[40,41]. We then purified the intracellular DNA fragments that accumulated in Trex1-deficient cells and devised a way to identify them by sequencing. We were surprised to find strong over-representation of DNA fragments that mapped to endogenous retroelements in Trex1-deficient cells compared to wild-type cells. We then showed that Trex1 potently blocks the retrotransposition of model endogenous retroelements by metabolizing reverse-transcribed cDNA[40]. On the basis of those findings, we proposed that the detection of retroelement cDNA by the innate immune system caused AGS and that the AGS enzymes function as antiretroviral proteins. It is important to note that at the time we identified the connections among the DNA-sensing pathway, the metabolism of retroelement cDNA and autoimmunity, very little was known about how infection with retroviruses is sensed by the innate immune system or whether a mechanism for detecting retroviruses in infected cells even exists[42]. As discussed below, several subsequent studies have confirmed and extended our findings in the context of infection with HIV and have established a new and exciting area of investigating the interactions between HIV and its human host.

The first description of a role for the sensing of cDNA during infection with HIV was provided by a study demonstrating that in addition to its

role in metabolizing the reverse-transcribed cDNA of endogenous retroelements, Trex1 could also degrade cDNA during infection with HIV[43]. Interestingly, cells depleted of Trex1 triggered a STING-dependent type I interferon response to infection with HIV. At almost precisely the same time, another group reported that infection of human dendritic cells with HIV-1 activated a potent, IRF3-dependent type I interferon response[44]. HIV-1 does not normally infect myeloid cells because of a failure to complete reverse transcription, so the authors used a 'trick' to enable productive infection: virus-like particles were included from simian immunodeficiency virus of macaques, which have an accessory factor (Vpx) that overcame the post-entry restriction of HIV reverse transcription[45]. Finally, infection of human lymphoid aggregate cultures with HIV-1 was found to cause massive depletion of abortively infected human $CD4^+$ T cells[46]. This cell-death response was caused by the accumulation of incomplete reverse-transcription intermediates. Together these three important papers established that HIV is indeed detected by the innate immune system, and they raised many important questions. How does the accessory protein Vpx enable the infection of otherwise refractory cells by HIV-1, and how is this tied to activation of the interferon response? What are the sensors of HIV-1 cDNA and how are they connected to interferons and cell death?

A key insight arrived several months later, when two groups identified SAMHD1, first described in 2009 as the product of a gene mutated in AGS[33], as the key myeloid HIV-1 restriction factor that is targeted

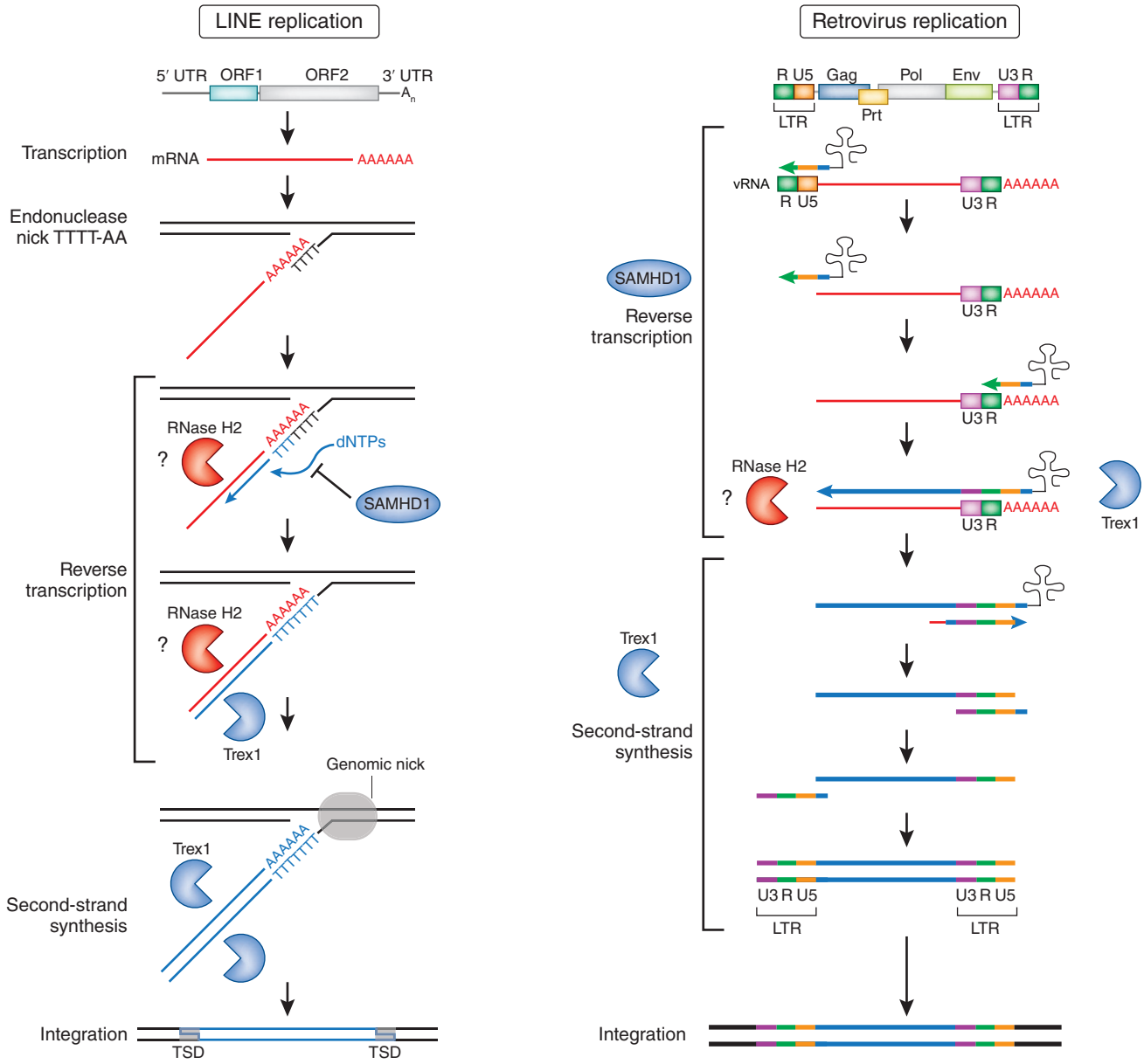

Figure 2 AGS-related enzymes restrict the replication of endogenous retroelements and exogenous retroviruses. In the replication of LINEs and retroviruses, AGS-related enzymes act throughout the steps of replication to restrict the products of reverse transcription. SAMHD1 inhibits reverse transcription through the degradation of cellular dNTPs. Trex1 metabolizes the DNA products of reverse transcription and can block the retrotransposition of endogenous retroelements. RNase H2 can degrade RNA of RNA-DNA hybrids and can also block the retrotransposition of endogenous retroelements, and we hypothesize that it acts by degrading viral RNA (vRNA) in the context of reverse transcription. U3, R and U5 denote regions of the retroviral LTRs. TSD, target-site duplication.

for degradation by Vpx[47,48]. SAMHD1 was then found to be a dNTP phosphohydrolase that functions to 'starve' the HIV reverse transcriptase of the nucleotides required for cDNA synthesis[49,50]. That function of SAMHD1 in preventing the reverse transcription of HIV extends beyond myeloid cells to resting CD4$^+$ T cells[51], which have long been known to be refractory to infection with HIV-1. Notably, the identification of the product of a second AGS-related gene as a potent antiretroviral enzyme provided crucial independent support for our model proposing that defective metabolism of retroelement cDNA causes AGS.

As it became clear that the detection of HIV by the innate immune system converges on its reverse-transcription intermediates, attention turned to identifying the relevant sensors of DNA involved in this detection. Many candidate receptors for intracellular DNA have been proposed, and considerable controversy remains about the relative contributions of those diverse proteins to the antiviral response[52]. The field enjoyed a major breakthrough with the discovery of the enzyme cyclic GMP-AMP synthase (cGAS)[53]. cGAS binds to immunostimulatory DNA and catalyzes the formation of cyclic GMP-AMP, which then binds to STING and triggers the interferon response[54]. The importance of that discovery cannot be overstated, and it has already spawned an entirely new area of investigation of the nature of the cyclic dinucleotide created by cGAS[55–57], as well as structural insight into the binding and catalysis of DNA by this enzyme[55,58–60]. Notably, cGAS is essential for the interferon response to DNA viruses, HIV and other retroviruses, and studies of this process have provided important evidence for the essential role of the cGAS–cyclic GMP–AMP–STING pathway in the DNA-activated antiviral response[61,62].

Interestingly, a study has found that the death of abortively infected CD4$^+$ T cells during infection with HIV is mediated by pyroptosis[63] dependent on the receptor IFI16 (ref. 64), a key sensor of DNA[65]. Moreover, IFI16 is essential for the interferon response to infection with HIV-1 in human monocytes[66]. Thus, cGAS and IFI16 have emerged as important sensors that mediate the type I interferon response to infection with HIV. Further work is needed to clarify the relative contributions of cGAS, IFI16 and other potential sensors of DNA to the immunological defense against HIV.

All of those remarkable studies have raised the important question of how that response to reverse-transcription intermediates of HIV was discovered only recently, given that scientists have been scrutinizing the immune response to infection with HIV for decades. One key reason is that HIV has evolved to avoid and manipulate detection by the innate immune system[67]. Indeed, the HIV accessory factor Vpu targets IRF3 for degradation[68,69] and thus severely blunts the DNA-activated antiviral response. Moreover, two studies have revealed that the HIV-1 capsid acts to specifically shield its reverse-transcription intermediates from being sensed by the innate immune system. Certain mutations in the genes encoding capsid proteins have revealed a potent, interferon-mediated response to infection with HIV; presumably these mutations act by destabilizing the structure of the HIV-1 capsid and allowing access of sensors in the innate immune system to the cDNA intermediates[70,71]. Those intriguing studies suggest that engineered mutations in the genes encoding capsid proteins or pharmacological inhibition of interactions between the capsid and its cellular partners may enable the design of a vaccine for HIV, based on the native virus, that efficiently stimulates a potent response by the innate immune system.

An evolutionary tradeoff in sensing DNA

The exciting studies described above have definitively proven that detection of HIV by the innate immune system indeed occurs in infected cells, that such detection is based mainly on the sensing of cDNA intermediates and that HIV has evolved to avoid and/or manipulate this antiviral response. Such findings have revealed a longstanding evolutionary 'arms race' between infectious retroviruses and their mammalian hosts. It is clear that this 'arms race' has affected the evolutionary trajectory of the APOBEC3 family of cytidine deaminases, the dNTP phosphohydrolase SAMHD1, the capsid-binding protein TRIM5α and other host restriction factors that target retroviruses[72,73]. We can therefore infer that similar pressures have shaped pathways for the sensing of intracellular DNA, with important implications for various autoimmune diseases. Specifically, we propose an evolutionary tradeoff with two opposing forces (**Fig. 3**). The first is the benefit of a more sensitive and robust response of the innate immune system to the cDNA of infectious retroviruses. That could in principle occur through higher expression of the sensors of DNA, through greater affinity of such sensors for DNA or through diminished function of key negative regulators that affect the DNA-activated antiviral response. More broadly, that selective pressure extends beyond retroviruses to include all viruses sensed by the same mechanism, including herpesviruses[65,74], adenoviruses[75] and presumably all other classes of DNA viruses. The evolutionary drive toward a more robust antiviral response is balanced by the need to avoid excessive responses to the cDNA of endogenous retroelements. We speculate that the requirement for minimal autoreactivity to retroelements places a limit on the sensitivity of the DNA-activated antiviral response (**Fig. 3**). Moreover, unlike the RIG-I-like receptors, which detect key structural features of viral RNA that are scarce in host RNA[10], all available structural evidence suggests that the key sensors of DNA simply detect double-stranded DNA independently of its sequence[58,76,77]. Thus, the limits on the sensitivity of the DNA-activated antiviral response are imposed not only by endogenous retroelements but also by the identical structures of foreign DNA and self DNA. Taking that one step further, we speculate that such unique evolutionary constraints may have enabled the development of DNA viruses that establish latency and lifelong infection of their hosts. In other words, the need to 'ignore' small amounts of retroelement cDNA may provide an opportunity, for example, for a herpesvirus to maintain a copy of its ~100- to 250-kilobase double-stranded DNA genome in cells without triggering a potent immune response (**Fig. 3**). Such latent DNA viruses probably actively inhibit detection by the innate immune system, which could further raise the threshold for sensing DNA in latently infected cells (**Fig. 3**). In contrast, just as there are no known endogenous RNA viruses, there are also no infectious RNA viruses that establish latency; RNA viruses are either cleared or establish chronic, symptomatic infection. We propose that this is because the RIG-I-like receptors are not constrained by the presence of abundant endogenous ligands, so they have evolved to be more sensitive than are the receptors of the innate immune system that detect DNA. Consequently, RNA viruses cannot persist below the threshold of detection by the innate immune system (**Fig. 3**).

Although we propose that the RNA-sensing pathway is more sensitive than the DNA-sensing pathway because of the lack of abundant endogenous RNA ligands, there are clear examples of autoimmunity associated with mutations in genes encoding RIG-I-like receptors, particularly Mda5 (refs. 78–80). Thus, a threshold exists for inappropriate activation of the sensors of RNA. Some of the autoimmunity-associated mutations in the gene encoding Mda5 confer constitutive basal activity but render the mutant receptor unresponsive to viral RNA ligands[80]; this suggests a ligand-independent mechanism for triggering autoimmune disease. In other cases, endogenous RNA ligands that are uncharacterized at present may exist that require removal to prevent autoimmune disease, similar to the mechanism by which Trex1 eliminates endogenous retroelement cDNA.

Interestingly, at least two products of AGS-related genes (Trex1 and SAMHD1) are positioned at the pivot point of that evolutionary tradeoff for sensing DNA (**Figs. 2** and **3**). Loss-of-function mutations in either of

those genes enables a more robust immune response to infectious retroviruses[43,44] but causes autoimmunity because of inappropriate detection of retroelement cDNA[40]. It would be very interesting to explore whether people who are heterozygous for mutations in the genes encoding those enzymes are protected from infection by DNA viruses and/or retroviruses. Such a benefit would provide a rationale for the existence and maintenance of those relatively rare alleles.

Retroelements and tissue-specific autoimmune disease

The hypothesis that defective metabolism of retroelement cDNA underlies AGS is in keeping with the clearly defined antiretroviral function of two of the AGS-related enzymes[40,43,47,48]. Moreover, we have similarly found that RNase H2, another AGS-related enzyme[32], potently restricts the retrotransposition of endogenous retroelements (H.E.V. & D.B.S., unpublished data). Thus, the products of five of the AGS-related genes can be placed in a common pathway of metabolism of retroelement cDNA intermediates (**Fig. 2**). In the framework of this model, there are two key questions that have not yet been adequately addressed. First, if each AGS-related enzyme can function as an antiretroviral enzyme, why are they not redundant with each other? Second, how can retroelements, which are ubiquitously present in the genomes of all of human cells, cause tissue-specific autoimmune disease if their cDNA is not metabolized?

We propose the following model for the interaction of AGS-related enzymes with retroelements (summary, **Fig. 2**). As described above, SAMHD1 starves reverse transcriptase of the dNTPs needed to generate cDNA, which prevents the formation of potentially immunostimulatory nucleic acids. We speculate that RNase H2 attacks the RNA strand of the RNA-DNA hybrid that is an essential intermediate of reverse transcription. Finally, Trex1 can metabolize the cDNA of retroelements and retroviruses. Why, then, do mutations in the genes encoding each individual enzyme cause AGS? For example, mutations in the gene encoding SAMHD1 would result in the production of more cDNA, but RNase H2 and Trex1 should still (in principle) be able to metabolize that cDNA. Moreover, SAMHD1 and Trex1 are encoded by interferon-inducible genes[33,40], so their expression should increase in response to activation of the ISD pathway to enable more efficient metabolism of accumulated cDNA. We envision two possible reasons for the lack of redundancy among AGS-related enzymes. First, each enzyme may contribute uniquely to disposal of nascent cDNA. Given the sensitivity of the detection of cDNA dictated by the evolutionary tradeoff model described above, mutation of the gene encoding each enzyme may increase the abundance of endogenous retroelement cDNA above the threshold of detection, which results in a chronic antiviral response (**Fig. 2**). Second, each AGS-related enzyme may be part of a linear pathway for cDNA metabolism such that the product of one enzyme is the substrate for the next. For example, SAMHD1 may need to be recruited to sites of early reverse transcription, and that in turn may be required for subsequent RNase H2 activity on the RNA-DNA hybrid. Trex1 may then require access to DNA that has been exposed as a result of RNase H2 activity. In this way, each enzyme may depend on the activity of the others for its function, and mutation of the gene encoding one enzyme may prevent the antiretroviral function of the others.

It is important to note that an alternative model exists in which mutations in AGS-related genes result in a chronic DNA-damage response[81]. The most compelling evidence for this model has been provided by studies demonstrating an essential role for RNase H2 in the removal of ribonucleotides that are misincorporated into genomic DNA during replication[82,83]. Mice deficient in *Rnaseh2b* suffer early embryonic death caused by massive genome instability[82,83]; that is partially relieved by simultaneous deletion of the tumor suppressor p53, which controls many aspects of the DNA-damage response[82]. Depletion of SAMHD1 in fibroblasts results in increased genomic DNA damage[84], a patient with AGS who has *SAMHD1* mutations developed chronic lymphocytic leukemia and recurrent somatic *SAMHD1* mutations have been found in patients with chronic lymphocytic leukemia who do not have AGS[85]. While such findings clearly show a link between AGS-related genes and the DNA-damage response, the mechanistic connections to the autoimmune disorder remain undefined. Indeed, the *Rnaseh2b*-null mice show no evidence of the aberrant type I interferon response present in Trex1-deficient mice[40,82] and, to a lesser extent, in SAMHD1-deficient mice[86,87]. Interestingly, the RNase H2 in patients with AGS is still enzymatically active[88], which suggests a dichotomy between null alleles and AGS-associated alleles of the genes encoding RNase H2. Moreover, it is unclear how chronic damage to DNA could lead to interferon-dependent autoimmune disease. For these reasons, we suggest that the detection of retroelement cDNA represents a more plausible scenario for autoimmune disease in this setting, although further work is needed to reconcile these two models.

The ubiquitous presence of retroelements in all nucleated cells raises the question of how defective metabolism of such elements could result in an autoimmune disease that has clear tissue specificity in mice and in humans[40,41,89,90]. We speculate that the tissue specificity of the autoimmune disease reflects tissue-specific expression of functional retroelements. Most retroelement sequences in the genome are silenced by epigenetic mechanisms that prevent their transcription; these include the specific recruitment of chromatin-remodeling factors that form heterochromatin on those sequences[15,16]. Because of that, retroelement activity is maintained at a very low level in most cells most of the time. As

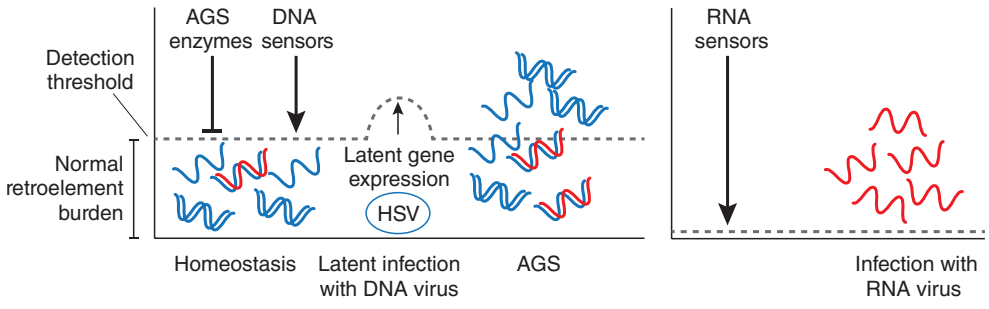

Figure 3 Model by which coevolution with endogenous retroelements shapes the DNA-sensing pathway. Low concentrations of nucleic acids arising from the reverse transcription of endogenous retroelements are kept in check by AGS-related enzymes. We predict that the sensitivity of the sensors of DNA (left) must be above that threshold to prevent inappropriate activation of the DNA-sensing pathway and autoimmune disease. That level of detection would help DNA viruses to maintain their genome in the nucleus during latent infection. We predict that during latency, some DNA viruses would act to inhibit the DNA-sensing pathway and thereby raise the threshold needed to initiate an immune response. During AGS, the burden of nucleic acids from endogenous retroelements reaches a concentration now detectable by the sensors of DNA and initiates a chronic immune response that leads to autoimmune disease. The threshold of sensitivity for sensors of RNA (right) can be much lower because of a lack of endogenous RNA ligands. HSV, herpes simplex virus.

mentioned above, there are 248 functional copies of the gene encoding L1 reverse transcriptase in the human genome, among thousands of non-functional genes encoding reverse transcriptase. However, tissue-specific expression of a functional retroelement could occur if that retroelement were present in the intron of an abundantly expressed, essential, tissue-specific gene. In this context, it will be very interesting to compare the genomic locations of all of the functional retroelements with an 'atlas' of the abundance of mRNA transcripts and genome-wide chromatin states in specific tissues. Such data sets are now available for many tissues. We predict that a limited number of sequences encoding functional retroelements will be found in tissue-specific genes that coincide with affected tissues in AGS and related diseases. Such an analysis is simpler for humans than for mice because mice have over ten times as many functional copies of the L1 reverse transcriptase[18], along with many functional endogenous LTR retroviruses that do not exist in humans.

If tissue-specific expression of retroelements exists, we can extend that model to include environmental stimuli that cause transient derepression of retroelements. Intriguingly, the DNA damage that results from exposure to ultraviolet light induces the massive transcription of retroelements and a substantial increase in cellular reverse-transcriptase activity[91,92]. If the expression of retroelement cDNA in such damaged cells exceeds the threshold for detection by sensors of DNA, an inflammatory response would be initiated, particularly in those cells with defective metabolism of the retroelement cDNA. *Trex1* mutations result in a monogenic form of cutaneous lupus called 'familial chilblain lupus' and are strongly associated with SLE[35–38], and photosensitivity to ultraviolet light is a common feature of SLE. We speculate that metabolism of retroelement cDNA is linked to those episodic cutaneous features and, more broadly, that environmental stimuli that affect retroelement expression may similarly drive inflammation through the same mechanism. This could be particularly relevant for the heterozygous mutations in *TREX1* that are strongly associated with SLE. Such mutations may change the threshold for the sensing of cDNA in a more subtle way than do the AGS-related mutations, such that an additional environmental stimulus would be needed to increase the abundance of endogenous DNA ligands above the threshold for activation of the aberrant antiviral response.

Implications for novel therapies

The identification of a role for the metabolism of retroelement cDNA in AGS, together with the delineation of the key pathways of the innate immune system that mediate autoimmunity, provides new opportunities for the development of novel therapeutic interventions for AGS, which is untreatable and incurable at present (**Fig. 4**). Over 25 years ago it was discovered that AGS is associated with elevated concentrations of type I interferons[31], and mouse models of AGS have confirmed the central pathogenic role of those cytokines in disease progression[40,41]. Biological agents that antagonize interferon signaling are in development and hold great promise for ameliorating the interfeon-dependent aspects of disease. Similarly, pharmacological inhibition of TBK1, the kinase that is essential for DNA-activated production of interferon[93–95], would blunt the chronic antiviral response. The identification of the cGAS–cyclic GMP–AMP pathway for the sensing of DNA offers a very appealing target for specific inhibition[53,54]. However, such approaches are not ideal because cGAS- and TBK1-dependent production of interferon is essential for antiviral immunity to many infectious viruses, so long-term treatment of patients with such drugs would probably result in greater susceptibility to infection. In the context of AGS, the severity of the disease may outweigh these potential complications[96], so such approaches should be considered as viable therapeutic options.

A novel approach to the treatment of AGS and related diseases is preventing the formation of the immunostimulatory nucleic acids in the first place. For cDNA from endogenous retroelements, twelve reverse-transcriptase inhibitors (RTIs) that are effective against HIV have been approved by the US Food and Drug Administration. A 'cocktail' of three of those RTIs has been found to rescue Trex1-deficient mice from death[97]. That important experiment has opened the door to a potential therapeutic approach that has four key advantages. First, RTIs would block the disease at its most proximal source by preventing the accumulation of immunostimulatory nucleic acids. Second, this treatment would be highly specific for AGS without the potential immunosuppressive effects of global blockade of interferon. Third, these drugs have already been approved by the US Food and Drug Administration, and many of them have produced years of clinical data detailing their safety, tolerability and side effects. Finally, unlike HIV, which can easily mutate to abrogate its sensitivity to a specific RTI, endogenous retroelements cannot rapidly evolve resistance to drugs because they are not infectious.

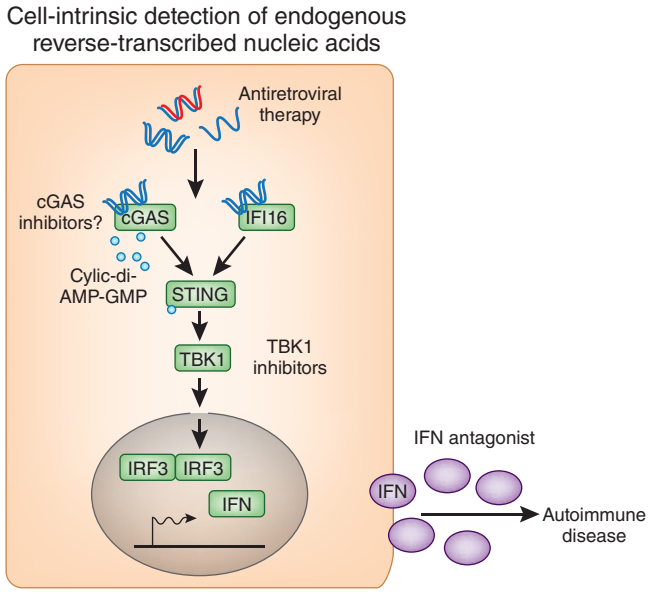

Figure 4 Therapeutic strategies for mitigating cell-intrinsic activation of the ISD pathway. The failure to metabolize reverse-transcription products causes inappropriate activation of the DNA-sensing pathway, chronic production of type I interferons and autoimmune disease. Disease-intervention strategies include the blockade of type I interferons, prevention of signaling via the ISD pathway through the use of TBK1-specific inhibitors, and elimination of reverse-transcription products through the use of RTIs. IFN, interferon.

Going forward, various key questions must be answered to delineate the potential utility of using RTIs to treat AGS and related diseases. Most importantly, which reverse transcriptase is relevant for disease? The L1 reverse transcriptase is the most likely candidate because hundreds of functional copies of the gene encoding this enzyme exist in the human genome and it can reverse-transcribe a variety of polyadenylated RNAs, including the L1 RNAs, SINE RNAs and certain cellular RNAs[15,16]. In contrast, there are no known endogenous LTR retroviruses that are fully functional in humans. That point is particularly important because the need to inhibit L1 reverse transcriptase would eliminate all non-nucleoside RTIs from consideration, as non-nucleoside RTIs specifically target HIV reverse transcriptase and related retroviral enzymes but are not effective against L1 (H.E.V. and D.B.S., unpublished data). The specific RTIs must not only target the appropriate reverse transcriptase enzyme(s) but also be able to access the relevant tissues and cells to inhibit the formation of cDNA *in situ*. In AGS, the brain is a prominent site of autoimmune attack. Thus, these drugs must be able to cross the blood-brain barrier. Finally, if we extend the possible utility of RTIs to SLE, a more common

autoimmune disorder, it will be important to know which patients with SLE could potentially benefit from this therapeutic approach. This would require a means of distinguishing patients with SLE who have chronic activation of the ISD pathway from those with disease driven by distinct mechanisms, such as hyperactive signaling via Toll-like receptors. If such stratification of patients with SLE were possible, this would go a long way toward 'personalizing' therapies on the basis of the underlying foundation of the autoimmune response.

In summary, we have outlined recent advances that have established detection of retroelement cDNA by the innate immune system as a major contributor to specific autoimmune diseases. We have proposed an evolutionary tradeoff between immunological detection of exogenous retroviruses and that of endogenous retroelements that places unique constraints on the sensing of foreign DNA. We have placed the AGS-related enzymes in the context of that model, and we suggest that RTIs hold considerable promise for the treatment of AGS and related diseases. Further understanding of the mechanisms that underlie those autoimmune disorders will undoubtedly reveal new and exciting avenues for the development of useful therapies to treat those diseases.

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The authors declare no competing financial interests.

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