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Mouse models for Aicardi–Goutières syndrome provide clues to the molecular pathogenesis of systemic autoimmunity

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Summary

Aicardi–Goutières syndrome (AGS) is a hereditary autoimmune disease which overlaps clinically and pathogenetically with systemic lupus erythematosus (SLE), and can be regarded as a monogenic variant of SLE. Both conditions are characterized by chronic activation of anti-viral type I interferon (IFN) responses. AGS can be caused by mutations in one of several genes encoding intracellular enzymes all involved in nucleic acid metabolism. Mouse models of AGS-associated defects yielded distinct phenotypes and reproduced important features of the disease. Analysis of these mutant mouse lines stimulated a new concept of autoimmunity caused by intracellular accumulations of nucleic acids, which trigger a chronic cell-intrinsic antiviral type I IFN response and thereby autoimmunity. This model is of major relevance for our understanding of SLE pathogenesis. Findings in gene-targeted mice deficient for AGS associated enzymes are summarized in this review.

Keywords: Aicardi–Goutières syndrome, interferon, nucleic acid sensing, SLE

Introduction

Systemic lupus erythematosus (SLE) is a prototypic systemic autoimmune disease characterized by autoantibodies to conserved nuclear antigens such as DNA, histones and ribonuclear proteins ('anti-nuclear antibodies'). Deposition of immune complexes in tissues is considered a key pathogenic event. Clinical presentation of the disease is characterized by considerable heterogeneity, and manifestations can include arthritis, dermatitis, nephritis and CNS inflammation. SLE patients typically feature chronic activation of the type I interferon (IFN) system [1,2], which most probably represents a critical event in the breakdown of self-tolerance [3–6]. A major trigger for type I IFN responses is recognition of viral nucleic acid by pattern recognition receptors of the innate immune system [7,8]. Importantly, discrimination between endogenous and microbial nucleic acids by mammalian innate sensors is not perfect, and excessive amounts of free self-nucleic acids or their appearance in compartments that are nucleic acid-free in the healthy organism can trigger anti-viral IFN responses in the absence of infection [9]. In SLE, genetic defects of mechanisms responsible for the disposal of apoptotic cells, cellular debris and nucleic acids play an important role in abnormal exposure of innate nucleic acids sensors to endogenous nucleic acids [10–12].

An important extension of the concept of pathogenic IFN responses triggered by endogenous nucleic acids came from research into the pathogenesis of the rare hereditary autoimmune disease Aicardi–Goutières syndrome (AGS). AGS manifests perinatally or in early childhood with encephalitis and severe neurological impairment [13,14]. The disease overlaps clinically and genetically with SLE [14–16], and can be regarded as a monogenic variant of SLE. In addition, mutations in three prime repair exonuclease 1 (TREX1) or SAM domain and HD domain-containing protein 1 (SAMHD1) have been shown to cause a rare cutaneous form of lupus [14,17–19]. AGS mimics intrauterine

viral infection and is characterized by chronic activation of the type I IFN system [14,20]. The syndrome can be caused by mutations in one of several intracellular enzymes, which are involved in nucleic acid metabolism; 3′ repair exonuclease 1 (TREX1) is the most abundant intracellular 3′–5′ DNA exonuclease displaying the highest activity on dsDNA with 3′ overhangs [21,22]. RNase H2 is a ribonuclease which digests the RNA moiety of DNA/RNA hybrids [23]. It also incises 5′ of a single ribonucleotide embedded in a DNA double helix [23,24]. SAMHD1 is a deoxyguanosine triphosphate (dGTP)-dependent deoxynucleotide-triphosphate (dNTP) triphosphohydrolase which cleaves dNTPs into inorganic triphosphate and nucleosides [25,26]. However, the interaction of SAMHD1 with nucleic acids [27,28] might reflect other important functional features. For example, SAMHD1 was reported to have 3′–5′ exonuclease activity and cleave ssDNA or ssRNA as well as the RNA strand of DNA/RNA hybrids [29]. Adenosine deaminase acting on RNA 1 (ADAR1) is an RNA-editing enzyme, which deaminates adenosine to inosine [30]. Targeted inactivation of the genes encoding TREX1, RNase H2 or ADAR1 in mice resulted in distinct phenotypes. Information obtained from the analysis of these mutants has greatly promoted our understanding of physiological functions of these enzymes as well as the pathogenesis of AGS and SLE.

Systemic type I IFN-driven autoimmunity in *Trex1*$^{-/-}$ mice

Morita *et al*. generated mice with complete loss of TREX1 (*Trex1*$^{-/-}$ mice) by replacing part of the coding sequence with a neomycin resistance cassette [31]. Animals homozygous for this mutation featured a reduced lifespan with a median survival of 6 months. Until then, TREX1 was regarded primarily as a repair enzyme with functions in DNA base excision repair [32,33]. Therefore, it came as a surprise that the cause of lethality was not increased mutation rates and malignant growth, but rather an inflammatory disease. Most animals died of spontaneous myocarditis and subsequent circulatory failure [31]. In a later study, histological analysis also revealed an increased incidence of inflammatory changes in multiple other organs of *Trex1*$^{-/-}$ animals [34]. The lethal inflammatory phenotype of *Trex1*$^{-/-}$ mice remained unexplained until Stetson and Medzhitov found that the condition was autoimmune in nature [35]. They showed that the animals develop autoantibodies reactive with determinants of murine heart and that the disease of TREX1-deficient mice could be prevented by crossing the animals onto a *RAG2*$^{-/-}$ lymphocyte-deficient background [35]. A later study reported an increased incidence of antinuclear antibodies in *Trex*$^{-/-}$ mice, which is typical of lupus-like systemic autoimmune disease [34]. Most importantly, Stetson and Medzhitov found a striking transcriptional signature of type I IFN-inducible genes in *Trex1*$^{-/-}$ heart tissue, suggesting that loss of TREX1 resulted in a spontaneous activation of an anti-viral type I IFN response [35]. Because chronic exposure to type I IFN can induce autoimmunity, the authors asked whether type I IFN was pathogenetically relevant in *Trex1*$^{-/-}$ mice and crossed them onto a type I IFN receptor-deficient (*IFNAR*$^{-/-}$) background. *Trex*$^{-/-}$*IFNAR*$^{-/-}$ mice were completely devoid of pathology demonstrating the pivotal role of type I IFN in the pathogenesis [35]. The finding that loss of a cytosolic nuclease triggered a spontaneous cell-intrinsic anti-viral IFN response stimulated the hypothesis that TREX1-deficiency results in accumulation of nucleic acids in the cytosol which are sensed by the cellular innate immune system [35].

This concept raised the question of which innate sensor pathways induced chronic IFN expression in *Trex1*$^{-/-}$ mice. Two transcription factors, IFN regulatory transcription factor (IRF)3 and IRF7, are essential for induction of type I IFN genes in response to viral infection. The constitutively expressed factor IRF3 is considered critical for the early IFN response to immunostimulatory DNA, while the IFN-inducible factor IRF7 was reported to amplify the response in later phases [36,37]. Stetson and Medzhitov found that additional knock-out of the *IRF3* gene prevented the pathology of *Trex1*$^{-/-}$ mice [35]. Gall *et al*. reported that deficiency for the adaptor molecule stimulator of IFN genes (STING) also resulted in rescue of the *Trex1*$^{-/-}$ phenotype [34]. Thus, via an unknown sensor, the putative nucleic acid accumulating in *Trex1*$^{-/-}$ cells seems to trigger the STING-dependent ISD-response pathway [37] resulting in IFN gene induction by IRF3.

Another important question is where and in which cell type the pathogenic IFN response occurs in *Trex1*$^{-/-}$ mice. Gall *et al*. visualized type I IFN action on IFN-responsive cells in *Trex1*$^{-/-}$ mice by crossing TREX1-deficiency to mice harbouring a type I IFN-inducible Cre transgene (Mx1–Cre) and a Cre-excision reporter allele (R26Y) [34]. A few days after birth, the majority of blood leucocytes had already been exposed to IFN. In the heart, the major target organ of autoimmunity in *Trex1*$^{-/-}$ mice, reporter-expressing cells were first observed in a circumscribed subendocardial area of the myocardium in neonatal mice. Experiments based on transplantation of wild-type or *IFNAR*$^{-/-}$ bone marrow into irradiated *Trex1*$^{-/-}$ recipients suggested non-haematopoietic cells as the primary source and haematopoietic cells as important targets of the pathogenic type I IFN [34]. Crosses of *Trex1*$^{-/-}$ mice to B cell- or T cell-deficient strains revealed that inflammatory pathology in most tissues depends upon T cells with no detectable role for B cells. Nevertheless, the glomerulonephritis of TREX1-deficient mice required a functional B cell system, and the absence of B cells delayed mortality of *Trex1*$^{-/-}$ mice significantly [34].

While the essential role of type I IFN in the pathogenesis of autoimmunity ensuing from loss of TREX1 is undisputed [35], induction of genes encoding type I IFN seems to be

restricted to particular cell types key to the pathogenetic chain. As shown by Hasan *et al.*, other cell types of *Trex1*$^{-/-}$ mice, including fibroblasts, also activate the STING/TBK1/IRF3-dependent cytosolic DNA sensing pathway and thereby induce a spectrum of anti-viral genes; however, in these cells anti-viral gene induction occurs in an IFN-independent manner [38]. In the same study, Hasan *et al.* also demonstrated that the late endolysosomal compartment is enlarged in *Trex1*$^{-/-}$ mice and that TREX1 functions to reduce mammalian target of rapamycin complex 1 (mTORC1) activity resulting in IRF3-mediated, IFN-independent induction of anti-viral genes as well as enhanced biogenesis of lysosomes. IFN-independent induction of anti-viral genes conferred relative resistance of TREX1-deficient cells to viral infection [38]. Whether or not this novel pathway also contributes to AGS and lupus pathogenesis remains to be determined.

Trex1$^{-/-}$ cells were found to display spontaneous DNA damage and chronic checkpoint activation [39], while this finding was not reproduced by others [35]. Whether loss of a function of TREX1 in DNA repair or genome maintenance resulting in a spontaneous DNA damage response contributes to pathogenesis in mice and humans with mutations in TREX1 is currently unclear.

In conclusion, TREX1-deficient mice have proved invaluable to shed light on the molecular pathogenesis of TREX1-associated AGS. Important questions to be answered include the following: (i) what is the nature of nucleic acids, which may accumulate in *Trex1*$^{-/-}$ cells and trigger the cellular innate anti-viral response; (ii) which sensors trigger the pathogenic IFN response; and (iii) why is the major target organ in AGS, the CNS, spared in murine TREX1-deficiency, which triggers autoimmunity in numerous other organs but not in the brain?

Genome instability due to increased genomic ribonucleotide load in RNase H2-deficient mice

Mammalian RNase H2 is a heterotrimeric complex composed of three subunits, RNase H2A, B and C, which are each encoded by a separate gene [40]. The A subunit carries the catalytic centre while the B and C subunits provide scaffolding and sites for protein interactions [23,41]. All three subunits of the yeast and human enzyme are required for activity *in vitro* [40,42] and AGS can be caused by mutations in any of the three genes [40]. The mutations found in AGS patients seem to reduce but not to abrogate activity of the enzyme, and bi-allelic null mutations in any of the three *Rnaseh2* genes have not been described in humans. This suggests that complete loss of RNase H2 activity results in early intrauterine lethality [40]. In accordance with this notion, mice that were completely devoid of RNase H2 activity died *in utero*. Targeted inactivation of RNase H2 subunits was performed independently by two different groups [43,44]. Reijns *et al.* generated mice carrying a premature stop in exon 7 of the *Rnaseh2b* gene, resulting in complete loss of RNase H2 activity (*Rnaseh2b*$^{-/-}$ mice). We generated *Rnaseh2c*$^{-/-}$ mice by replacing the complete coding region of the gene by a resistance cassette. Both groups also used *Rnaseh2b* 'knock-out first' (*Rnaseh2b*^{KOF}) mice [European Conditional Mouse Mutagenesis Program (EUCOMM), see below] to obtain mice with ubiquitous Cre-mediated deletion of exon 5, resulting in a frame shift and absence of RNase H2B protein. All these mutations led to complete loss of RNase H2 activity and resulted in similar embryonic lethality commencing on approximately embryonic day 9·5 (E9·5) [43,44]. Mutant embryos appeared normal at E6·5, but were reduced in size at E7·5 compared to controls [44]. At E9·5 massive developmental defects became evident and mutant embryos all died before E11·5 [44]. The *Rnaseh2b*^{KOF} mutation, an insertion of a splice acceptor followed by a translational stop element into introns 4–5 of the *Rnaseh2b* gene, was designed to completely prevent expression [45]. However, we found that this mutation represents a hypomorphic allele with normal splicing from exon 4 to exon 5 occurring at a low rate, resulting in low residual enzyme activity [43]. *Rnaseh2b*$^{KOF/KOF}$ embryos featured delayed lethality compared to embryos with complete deficiency for the enzyme, were smaller than controls at E14·5 and died perinatally [43]. Histological analysis of stillborn *Rnaseh2b*$^{KOF/KOF}$ mice revealed proportionally small organs and no inflammatory changes that could explain lethality [43]. RNase H2-deficient embryos and cell lines grown from such embryos showed reduced proliferation compared to control embryos and cells [43,44]. In order to elucidate the reasons for proliferative defect and early lethality, global gene expression profiles of whole E9·5 *Rnaseh2b*$^{-/-}$ embryos and E14·5 *Rnaseh2b*$^{KOF/KOF}$ fetal liver cells were investigated by Reijns *et al.* and ourselves, respectively, with remarkably similar results [43,44]. While no expression signatures indicative of inflammatory or type I IFN responses were detected, several p53-inducible genes were found up-regulated in mutant *versus* control samples, including the cell cycle inhibitor p21 encoded by the *Cdkn1a* gene. This finding suggested that compromised RNase H2 activity might lead to DNA damage and thereby cause cell cycle arrest.

Reijns *et al.* stained E6·5 *Rnaseh2b*$^{-/-}$ embryos for histone H2A.X phosphorylated at serine 139 (pH2AX), which marks DNA double-strand breaks, and found high numbers of pH2AX foci in mutant but not control epiblast cells [44]. We detected increased numbers of pH2AX foci in E18·5 *Rnaseh2b*$^{KOF/KOF}$ thymocytes and liver cells compared to control cells [43]. On a p53-deficient background, a partial rescue of the *Rnaseh2b*$^{-/-}$ embryonic phenotype and the proliferative impairment of *Rnaseh2b*$^{-/-}$ cells were observed [44]. *Rnaseh2b*$^{-/-}$*p53*$^{-/-}$ cells displayed genomic instability with frequent occurrence of micronuclei and chromosomal rearrangements [44]. These findings suggested strongly that the lethal phenotype of RNase

H2-deficient embryos was caused by a spontaneous DNA damage response.

Nick McElhinny *et al*. demonstrated genomic instability in RNase H2-deficient yeast due to increased numbers of ribonucleotides contained in the genomic DNA [46]. The high ribonucleotide load of mutant yeast DNA was reflected in enhanced sensitivity to alkaline hydrolysis. This finding suggested that RNase H2 functions to remove ribonucleotides misincorporated by replicative polymerases. Reijns *et al*. found that DNA from *Rnaseh2b*$^{-/-}$ E9·5 embryos, like the mutant yeast DNA, featured increased alkali sensitivity [44]. We used the specificity of prokaryotic RNase HII, which incises DNA at ribonucleotides embedded in the double helix, for ribonucleotide detection in DNA from RNase H2-deficient E9·5 embryos. Nicks introduced by the bacterial enzyme were visualized by nick translation-based incorporation of radioactive label [43]. This approach also revealed a dramatic increase in the ribonucleotide load of mutant genomic DNA. Based on the lengths of alkaline hydrolysis fragments, Reijns *et al*. determined that the DNA from RNase H2-deficient embryos contained one ribonucleotide per 7600 base pairs (bp). If not removed, misincorporated ribonucleotides therefore represent the most common lesion in mammalian DNA [44].

Ribonucleotide accumulation in DNA from RNase H2-deficient mice probably originate from misincorporation by replicative DNA polymerases, which was shown to be a frequent event in yeast [47]. Incomplete removal of Okazaki fragment RNA primers or spontaneous oxidation of DNA [48] might also contribute to the genomic ribonucleotide content. The presence of high numbers of ribonucleotides in DNA can cause DNA damage by several different mechanisms. Spontaneous hydrolysis at two neighbouring ribonucleotides on opposite strands might result in double-strand breaks. Ribonucleotides in genomic DNA were also shown to cause replication fork stalling in yeast [49]. Furthermore, topoisomerase-I was reported to cleave double-stranded DNA containing single ribonucleotides [50]. Topoisomerase-I-mediated cleavage of DNA entails the transient formation of a covalent bond of the enzyme to 5′ end. Presence of widespread topoisomerase-I cleavage complexes may result in their frequent collision with replication forks and the formation of double-strand breaks [51]. Moreover, topoisomerase-I-mediated cleavage of DNA substrates containing a single ribonucleotide can result in formation of a 2′,3′-cyclic phosphate end [50,52], the processing of which could lead to manifestation of strand breaks.

An intriguing question is whether accumulation of ribonucleotides in genomic DNA, genomic instability and a chronic DNA damage response could contribute to the pathogenesis of AGS and related human disorders. Repair of genomic ribonucleotides in the absence of RNase H2 might produce aberrant nucleic acid species responsible for activation of the IFN system in the human. Genome-wide transcriptome profiling of RNase H2-deficient embryos, however, did not reveal a signature of IFN-inducible genes [43,44]. Inflammatory changes indicative of autoimmune disease were not observed in stillborn *Rnaseh2b*$^{KOF/KOF}$ neonates and crosses to *IFNAR*$^{-/-}$ or lymphocyte-deficient backgrounds did not improve survival [43]. Thus, cell-intrinsic activation of an anti-viral innate response, as observed in *Trex1*$^{-/-}$ and *Adar1*$^{-/-}$ animals (see below), does not occur in the RNase H2 mutant models analysed so far. IFN-mediated autoimmune pathology reflecting potential additional functions of RNase H2 in the removal of endogenous nucleic acids species might occur at later stages of ontogeny in mouse models of less severe or conditional RNase H2 deficiency, which bypass early lethality. Less severe reduction of RNase H2 activity in AGS patients compared with the current mouse models could be the reason that a DNA damage-related phenotype dominates in these mice, while RNase H2-associated AGS is characterized prominently by autoimmunity. Importantly, spontaneous DNA damage and chronic checkpoint activation were also observed in *Trex1*$^{-/-}$ cells [39].

Loss of ADAR1 in mice causes embryonic lethality associated with spontaneous induction of type I IFN-inducible genes

ADAR1 is a hydrolase that deaminates adenosine to inosine ('A-to-I editing') in dsRNA [30]. In man and mouse, two isoforms are transcribed from at least two different promotors. Whereas the shorter isoform (p110) is expressed constitutively, the full-length protein, p150, is inducible by types I and II IFNs [53,54]. The isoforms differ in their subcellular localization, probably reflecting distinct physiological functions [55,56]. While mutations of the *Adar1* gene were demonstrated only recently to cause AGS [20], various mouse models for ADAR1 deficiency were already described several years ago. Chimeric embryos with a high contribution of *Adar1* mutant cells died *in utero* due to defective erythropoiesis [57]. Using Cre-loxP technology, Wang *et al*. and Hartner *et al*. succeeded in the generation of ADAR1-deficient mice and found that embryos homozygous for the defective allele displayed embryonic lethality associated with increased rates of apoptosis in the liver [58,59]. Consistent with early lethality of *Adar1*$^{-/-}$ embryos, mutations identified in AGS patients were mainly missense variants predicted to result in reduction rather than complete loss of enzymatic activity [20]. Hartner *et al*. and XuFeng *et al*. demonstrated an essential role of ADAR1 in haematopoietic precursor cells [60,61] and in haematopoietic stem cells [60]. Hartner *et al*. performed global transcriptome analysis of *Adar1*$^{-/-}$ fetal liver haematopoietic stem cells and found a striking up-regulation of type I IFN-inducible genes. A similar tran-

scriptional signature of type I IFN-inducible genes was found in cells of the erythroid lineage and in non-haematopoietic tissues of E11 *Adar1*$^{-/-}$ embryos. Transcription of one of the up-regulated genes most strongly in ADAR1-deficient haematopoietic stem cells, *Stat1*, was not enhanced in *Adar1*$^{-/-}$ myeloid cells, suggesting that spontaneous activation of the type I IFN system in response to loss of ADAR1 might be a cell type-specific phenomenon. Among the induced genes, several genes encoding RNA binding proteins [Toll-like receptor (TLR)-3, protein kinase R (PKR), retinoic acid-inducible gene 1 (RIG-I)] could be identified, hinting at an accumulation of a RNA species that might trigger the IFN production in ADAR1-deficient cells [60]. The transcriptional signature of ADAR1-deficient cells correlated with the signature of IFN-inducible genes found in cells expressing a variant of the dsRNA binding protein NF90 [62]. As ADAR1 was shown to interact with NF90 [63], this finding could reflect the involvement of both proteins in the same IFN regulatory pathway. In addition to its dsRNA binding domain, ADAR1 also possess a Z-DNA binding domain in the N-terminal part [64], potentially pointing to a mechanism of IFN induction that might be independent of the RNA-editing function of ADAR1. Functional dissection of the two ADAR1 isoforms, IFN-inducible p150 and constitutively expressed p110, could be instrumental in the elucidation of the suppression of an IFN response by ADAR1. However, a first attempt to abrogate expression of the IFN-inducible p150 isoform selectively by gene targeting [65] remains controversial [66,67].

In conclusion, like *Trex1*$^{-/-}$ mice, ADAR1-deficient animals are characterized by spontaneous IFN production and thus reproduce an important feature of humans carrying ADAR1 mutations. As in the case of *Trex1*$^{-/-}$ mice, urgent questions include whether an abnormal accumulation of nucleic acids is the trigger of the IFN response and which signalling pathways are responsible for the induction of IFN genes.

A unifying concept of AGS pathogenesis?

According to a fascinating concept that could explain spontaneous IFN release, the AGS-associated enzymes might restrict the activity of endogenous retroviruses or retrotransposons. This idea was first proposed by Stetson and Medzhitov based on experiments in TREX1-deficient mice [35]. Intriguingly, AGS-associated enzymes were shown to affect the replication of exogenous retrovirus. TREX1 degrades non-productive by-products of reverse transcription [68] and prevents their sensing by cellular pattern recognition receptors and induction of an anti-viral IFN response. Thus, TREX1 serves to hide HIV from the innate immune system. ADAR1 was reported to promote HIV replication [69,70]; however, anti-retroviral effects of ADAR1 were demonstrated in a recent study [71]. SAMHD1 potently suppresses HIV replication in non-cycling human cells by degrading dNTPs and reducing their concentration below a threshold required for viral reverse transcription [25,26,72–74]. Thus, defects of enzymes affecting retroviral replication result in spontaneous activation of type I IFN responses. However, despite this link of AGS-associated enzymes to viral control, and despite the clinical and biochemical similarities of AGS to congenital viral infection, no causal role of an exogenous retrovirus in AGS could be established. Therefore, it is tempting to speculate that replication intermediates of germline-encoded fossil retroviruses or retrotransposons could accumulate in the absence of the AGS-associated enzymes. Their sensing by intracellular pattern recognition receptors might trigger the pathogenic IFN response and lead to autoimmunity. In support of this concept, Stetson *et al.* detected retroelement-encoded DNA in the cytosol of *Trex1*$^{-/-}$ *heart* cells and observed enhanced activity of retrotransposition reporter elements *in vitro* [35], thus providing evidence for enhanced activity of retroelements in the absence of TREX1. Importantly, systemic administration of HIV-1 reverse transcriptase inhibitors ameliorated the pathology of *Trex1*$^{-/-}$ mice significantly [75], suggesting that inhibition of retroelement activity might represent a therapeutic approach in human autoimmune disease.

In addition to replication intermediates of endogenous retroelements, nucleic acids originating during DNA replication or repair could accumulate in cells lacking AGS-associated enzymes, and were discussed as the triggers of autoimmunity. Yang *et al.* found that the cytoplasmic compartment of *Trex1*$^{-/-}$ cells stained with an anti-ssDNA antibody and detected an ssDNA oligonucleotide of 60–65 bases in mutant but not control cells [39].

Perspective

Significant differences in the molecular response to inflammatory stimuli between mouse and man, as highlighted recently [76], limit modelling of human disease in the mouse. However, despite enormous interspecies differences, *Trex1*$^{-/-}$ and *Adar*$^{-/-}$ mice reproduce spontaneously the unbalanced type I IFN production of SLE and AGS patients with defects in these genes. These findings point to a pathogenic IFN-inducing principle that is conserved in evolution and is therefore of fundamental relevance. Defects of the enzymes TREX1, RNase H2, ADAR1 and SAMHD1 can all cause AGS. It will be crucial to clarify whether or not the four enzymes all function in the same pathway or rather in different pathways to prevent spontaneous IFN production. The AGS-associated enzymes might, for example, all prevent the accumulation of the same pathogenic nucleic acid species. Alternatively, different nucleic acids originating from different processes could accumulate as a result of compromised activity of each of the four enzymes. We have recently generated SAMHD1-deficient mice and are currently analysing these mutants for spontaneous activation

of anti-viral innate immunity. Identification of the nucleic acid species triggering autoimmunity in AGS remains an important task that will contribute significantly to our understanding of molecular mechanisms that cause SLE.

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Disclosure

The authors declare that there are no conflicts of interest.

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