

Aicardi-Goutières syndrome and related phenotypes: linking nucleic acid metabolism with autoimmunity

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Aicardi-Goutières syndrome (AGS) is a genetically determined encephalopathy demonstrating phenotypic overlap both with the sequelae of congenital infection and with systemic lupus erythematosus (SLE). Recent molecular advances have revealed that AGS can be caused by mutations in any one of five genes, most commonly on a recessive basis but occasionally as a dominant trait. Like AGS, SLE is associated with a perturbation of type I interferon metabolism. Interestingly then, heterozygous mutations in the AGS1 gene *TREX1* underlie a cutaneous subtype of SLE-called familial chilblain lupus, and mutations in *TREX1* represent the single most common cause of monogenic SLE identified to date. Evidence is emerging to show that the nucleases defective in AGS are involved in removing endogenously produced nucleic acid (NA) species, and that a failure of this removal results in activation of the immune system. This hypothesis explains the phenotypic overlap of AGS with congenital infection and some aspects of SLE, where an equivalent type I interferon-mediated innate immune response is triggered by viral and self NAs, respectively. The combined efforts of clinicians, geneticists, immunologists and cell biologists are producing rapid progress in the understanding of AGS and overlapping autoimmune disorders. These studies provide important insights into the pathogenesis of SLE and beg urgent questions about the development and use of immunosuppressive therapies in AGS and related phenotypes.

INTRODUCTION

A disturbance of interferon alpha (IFN- α) homeostasis is central to the pathogenesis of the prototypic autoimmune disorder systemic lupus erythematosus (SLE) (1–3). As in lupus, perturbation of IFN- α metabolism is a major pathogenic feature of the inflammatory encephalopathy Aicardi-Goutières syndrome (AGS) (4). In keeping with this, some children with AGS develop an early-onset form of SLE (5–8); heterozygous mutations in the *AGS1* gene *TREX1* underlie a cutaneous subtype of SLE-called familial chilblain lupus (FCL) (9); and, remarkably, ~2% of SLE patients harbour pathogenic mutations in *TREX1* (10). Rare but high-penetrant causes of lupus are important to identify because they provide immediate insights into pathogenesis.

TREX1 deficiency results in the intracellular accumulation of DNA and a type I interferon response accrues from the

activation of a Toll-like receptor (TLR)-independent pathway (11,12). Distinct from defects in central and peripheral lymphocyte tolerance or activation of non-cell-autonomous innate immune recognition through TLRs, these studies define a new mechanism for the initiation of autoimmunity by interferon-stimulatory nucleic acid (NA).

In this report, we provide a selected overview of the important clinical and molecular features of AGS and related phenotypes, and discuss recent data linking disordered NA metabolism with autoimmunity.

MOLECULAR BASIS OF AGS AND RELATED PHENOTYPES

In its classic presentation, AGS can be considered as a Mendelian mimic of the sequelae of *in utero* viral infection

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Table 1. Genes so far identified to be associated with the AGS phenotype

Gene	Locus	Protein	% of families with mutations
<i>AGS1</i>	3p21	TREX1/DNaseIII	35
<i>AGS2</i>	13q14	RNASEH2B	45
<i>AGS3</i>	11q13	RNASEH2C	15
<i>AGS4</i>	19p13	RNASEH2A	<5
<i>AGS5</i>	20q11	SAMHD1/DCIP	~10

(13). Severe neurological dysfunction becomes apparent in infancy, and manifests as progressive microcephaly, spasticity, psychomotor retardation and, in ~40% of cases, death in early childhood (14). Intracranial calcification, white matter destruction and brain atrophy are evident on neuroimaging, similar to the radiological findings seen in congenital infections, while outside the nervous system, thrombocytopenia, hepatosplenomegaly, elevated hepatic transaminases and intermittent fevers also erroneously suggest an infective process (15).

AGS is a genetically heterogeneous disorder caused by mutations in any of the genes encoding the 3'→5' exonuclease TREX1 (*AGS1*) (16), the three non-allelic components of the RNASEH2 endonuclease complex (*AGS2*, 3 and 4) (17) and the uncharacterized SAMHD1 protein (*AGS5*) (18) (Table 1). Evidence of further genetic heterogeneity exists.

AGS-RELATED GENES AND PROTEINS

TREX1

TREX1 (DNase III) represents the major 3'→5' DNA exonuclease activity measured in mammalian cells (19). It was originally thought that TREX1 may serve to excise mismatched dNTPs during lagging strand synthesis or gap-filling during base excision repair (20). However, TREX1 null mice did not show an increased spontaneous mutation frequency (21). Instead, like AGS patients, *TREX1* knock-out mice exhibit an inflammatory phenotype although, in contrast to AGS patients, they do not demonstrate neurological involvement but show an inflammatory cardiomyopathy (21). There is evidence to suggest that TREX1 associates, perhaps through its polyproline II helix (22,23), with the SET complex, a caspase-independent cell death pathway also including the DNA binding protein HMGB2 and the endonucleases APE1 and NM23-H1, where TREX1 might rapidly degrade 3' ends of nicked dsDNA (24). The significance of this possibility in relation to human disease is unclear.

The majority of recessive AGS-causing *TREX1* mutations are predicted as null alleles (with the recurrent missense transition c.341G>A involving the arginine residue at 114 crucial for protein dimerization and so likely also abrogating protein function) (14). Following the identification of AGS-causing biallelic mutations in *TREX1* in 2006, heterozygous *TREX1* mutations were described in a cutaneous form of SLE-called FCL (9,25,26), in rare cases of dominantly inherited AGS (9) and in patients with an adult-onset cerebro-retinal microangiopathy associated with Raynaud's phenomenon [retinal vasculopathy with cerebral leukodystrophy (RVCL)] (27). Furthermore, in light of the phenotypic and biochemical

overlap of AGS with lupus, heterozygous *TREX1* mutations were searched for and found in ~2% of a cohort of individuals with SLE (10) (Table 2).

The pathogenicity of, and phenotypic variability associated with, heterozygous *TREX1* mutations is explained, at least partly, by the residues involved and the position of the mutation (Fig. 1).

In particular, all RVCL-, and most lupus-, associated mutations cluster in the C-terminus, responsible for tethering the protein in the endoplasmic reticulum (10,27). Thus, C-terminus RVCL and lupus frameshift mutations have been shown to result in cellular mislocalization rather than a loss of enzymatic activity *per se* (although disturbed localization might effectively equate with an absence of enzymatic activity in the right place). Meanwhile, the heterozygous mutations at D18 and D200 (published in association with dominant FCL and *de novo* dominant AGS respectively) involve key catalytic residues which, *in vitro*, have been shown to possess significantly reduced exonuclease activity against double-stranded DNA (dsDNA) and to inhibit wild-type protein activity (30). It is of note that some AGS patients have C-terminus frameshift mutations, and there is considerable overlap of the missense mutations reported in lupus patients (including the R114H mutation identified on more than 60 AGS-associated disease alleles) with those seen in AGS (14). These observations suggest that some AGS patients and their heterozygous parents may be at risk of developing RVCL and SLE.

RNASEH2

Ribonucleases H (RNASEH) are endonucleases that cleave the RNA of RNA/DNA hybrids in a sequence non-specific manner (31). Eukaryotes have two types of RNASEH (H1/I and H2/II) showing distinct enzymatic and site-specific activity with RNASEH2 enzymes able to recognize single ribonucleotides embedded in DNA duplexes (32,33). RNASEH2 is the major source of cellular ribonuclease activity in eukaryotes (33,34). Eukaryotic RNASEH2 is composed of three different proteins, the catalytic subunit (2A), and two further subunits (2B, 2C) (encoded by *AGS4*, *AGS2* and *AGS3*, respectively) that have no prokaryotic counterparts and as yet unknown functions but that are necessary for catalysis (17,35,36).

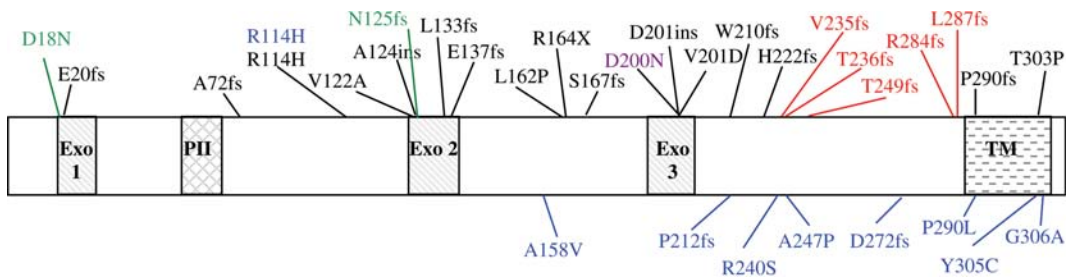
In contrast to *TREX1*, the overwhelming majority of *AGS2*, 3 and 4 mutations are hypomorphic, and it is of note that biallelic null mutations in any of these genes have never been observed (14), suggesting that such a state may be lethal or result in presently unrecognized phenotypes. In keeping with this, recent work demonstrates that most *RNASEH2* complex mutations so far studied do not alter recorded nuclease activity (36,37). The failure to note differences in activity *in vitro* suggests that changes in assembly, stability or localization of the complex might be important in producing a disease phenotype.

The ability of RNASEH2 to recognize and cleave a single ribonucleotide in a DNA duplex suggested a possible role for the enzyme in DNA repair where DNA polymerases might mistakenly incorporate a ribo- rather than deoxyribonucleotide (38). Other proposed functions are in the suppression of R-loops transiently formed during transcription (31,39), and the hydrolysis of Okazaki fragment RNA primers (40). Recent data indicate that RNASEH2B may interact with

Table 2. Summary of recognized phenotypes associated with *TREX1* mutations

Phenotype	AGS	RVCL	FCL	SLE
Inheritance	AR (rare AD cases)	AD	AD	Rare monogenic forms
Genes	<i>TREX1</i> (9,16), <i>RNASEH2A/B/C</i> (17), <i>SAMHD1</i> (18)	<i>TREX1</i> (27)	<i>TREX1</i> (9,25,26)	Monogenic: <i>TREX1</i> (10), <i>DNASE1</i> (28), complement deficiency (29)
Onset	Prenatal—usually <12 months	30–50 years	Childhood	Usually 15–40 years
Mortality	40% <10 years of age	5–20% 10 year mortality (from onset)	Non-lethal	5–20% 10 year mortality
Neurological involvement	Severe intellectual and physical disability	Strokes, seizures, migraine, cognitive decline	None	Neuro-lupus: strokes, seizures, psychosis, cognitive decline

References shown in brackets.



Mutations: Black = recessive AGS; Purple = dominant AGS; Green = FCL; Blue = SLE; Red = RVCL. Exo1, 2, 3 domains; PII, polyproline II domain; TM, Transmembrane domain

Figure 1. Schematic of (selected) disease-associated *TREX1* mutations. Mutations: black, recessive AGS; purple, dominant AGS; green, FCL; blue, SLE; red, RVCL. Exo1, 2, 3 domains; PII, polyproline II domain; TM, transmembrane domain.

proliferating cell nuclear antigen (PCNA), via a PCNA-interacting peptide at its C-terminus (36), a protein essential for eukaryotic Okazaki fragment processing during lagging strand synthesis.

SAMHD1

This summer, mutations in the *AGS5* gene encoding SAMHD1 were reported to cause AGS (18). As for *TREX1*, mutations in *SAMHD1* include biallelic null alleles as well as missense mutations. The functions of the 626 amino acid protein SAMHD1 are currently unknown. SAMHD1 was originally identified in a human dendritic cell cDNA library as an orthologue of the mouse IFN- γ -induced gene *Mg11* (41), hence the alternative name dendritic cell-derived IFN- γ -induced protein (DCIP) (NB. *Mg11* was cloned by Lafuse *et al.* (42) although the cited reference discusses an unrelated gene *Mg21*). Other evidence also implicates SAMHD1 in immunity since it is upregulated in response to viral infections (43–45) and may have a role in mediating TNF- α proinflammatory responses (46). The SAMHD1 name derives from the presence of a sterile alpha motif (SAM) and a HD domain in tandem, an arrangement which is apparently unique amongst human proteins. SAMs are 65–70 residues in length and can serve as protein-interaction modules mediating interactions with other SAM domain and non-SAM domain-containing proteins (47). Additionally, the SAM domains of *S. cerevisiae* Vts1p and its *D. melanogaster* homolog Smaug bind an RNA stem-pentaloop hairpin in a sequence non-specific manner (48). The HD domain, characterized by a doublet of

divalent-cation-coordinating His and Asp residues, is found in a diverse superfamily of enzymes with predicted or known phosphohydrolase activity (49). It is noteworthy that nucleotides are the substrates of five HD-domain enzymes characterized to date (50), while a sixth, YhaM, has a known exonuclease activity (51).

PATHOGENESIS

Two recent high-profile papers on *TREX1* have provided crucial insights into the pathogenesis of AGS. In 2007, Yang *et al.* (11), using *TREX1* $-/-$ mouse embryonic fibroblasts and AGS-derived patient fibroblasts, corroborated previous data to show that *TREX1* predominantly localizes to the cytoplasm. They also demonstrated a relocation of the protein to BrdU positive foci in the nucleus during S phase and presented data suggesting that *TREX1* null cells exhibit defective G1/S transition and chronic ATM-dependent checkpoint protein activation. Remarkably, *TREX1* null cells were shown to accumulate 60–65 nucleotide long single-stranded DNA (ssDNA) species proposed to derive from cells in S phase. Taken together, these data were interpreted to indicate that *TREX1* acts on ssDNA polynucleotides, generated from the processing of replication intermediates, to attenuate checkpoint signalling and prevent pathological immune activation. Considering a possible common substrate on which both *TREX1* and the *RNASEH2* complex might act, the authors invoked a model involving the ‘folding back’ of short flaps of DNA with an attached RNA primer, produced by the resolution of Okazaki fragments during lagging strand synthesis.

Table 3. Summary of mouse cross data presented by Stetson *et al.* (12)

Mouse model	Phenotype and IFN status
TREX1 null	Inflammatory cardiomyopathy: high IFN
TREX1/IRF3 DKO	'Cured': low IFN
TREX1/IFN α R1 DKO	'Cured': low IFN
TREX1/RAG2 DKO	'Cured': high IFN

DKO, double knock-out; IRF3, interferon regulatory factor 3; IFN α R1, type I IFN-receptor.

Having previously defined the IFN-stimulatory DNA (ISD) response, a cytosolic antiviral pathway that detects DNA, in 2008 Stetson *et al.* (12) showed that TREX1 is an essential negative regulator of the ISD response. Using a series of mouse crosses to dissect the pathway linking TREX1 deficiency to lethal autoimmunity, they demonstrated a TLR-independent pathway signalling through the transcription factor IRF3 (Table 3). As expected from human studies, an intact type I IFN response was necessary to develop the disease phenotype. Of considerable interest, by crossing the TREX1 null mouse with a knock-out for the DNA recombinase RAG2 required for generating functional lymphocytes, they also showed that the inflammatory pathology was dependent on antibody production (Table 3). Controversially, unlike Yang *et al.*, Stetson *et al.* (12) found no evidence for the activation of DNA damage checkpoint signalling, which they suggested might be an artefact related to cell line immortalization. Rather, although they also demonstrated an accumulation of ssDNA in TREX1 null cells, they suggested that such DNA derived from endogenous retroelements otherwise metabolized by functional TREX1.

Nucleic acid metabolism in AGS

As TREX1 and RNASEH2 are nucleases, it was previously hypothesized that these proteins might remove 'waste' NAs, and that a failure of this process could result in immune activation (17,52). The work of both Yang *et al.* (11) and Stetson *et al.* (12) shows that TREX1 deficiency does indeed lead to the intracellular accumulation of DNA, and the data generated by Stetson *et al.* further demonstrate activation of the immune system by these accumulated NA (12). Distinct from defects in central and peripheral lymphocyte tolerance or activation of non-cell-autonomous innate immune recognition through TLRs, these studies define a novel cell-autonomous, TLR-independent mechanism for the initiation of autoimmunity by IFN-stimulatory NA (Fig. 2).

Nucleic acids and autoimmunity

The innate immune system detects viral infections, induces antiviral effectors that neutralize the spread of infection and activates antigen-specific adaptive responses (53). Type I interferons play an important role in the coordination of this response. In many cases, the presence of virus is detected by receptors that recognize viral NA. TLRs are transmembrane proteins localized at the cell membrane or in endoplasmic compartments of specialized immune cells. TLR-3, -7/8 and

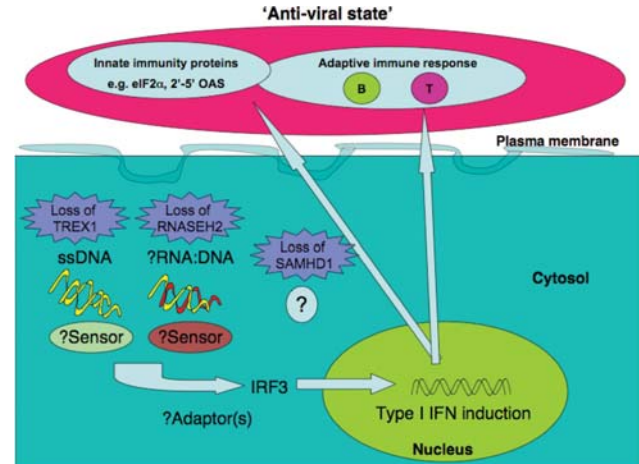


Figure 2. Model of disease pathogenesis in deficiency of TREX1, RNASEH2 and SAMHD1 activity. NA (ssDNA in TREX1 deficiency putatively derived from endogenous retroelements (12) and/or Okazaki fragments (11); possibly RNA:DNA hybrids in absence of RNASEH2 (17); unknown in SAMHD1 deficiency (18)) accumulate and are recognized by as yet undefined sensors leading to the TLR-independent induction of type I IFN via the transcription factor IRF3. At least in TREX1 deficiency, functional lymphocytes are necessary to propagate the disease phenotype (12).

-9 recognize viral double-stranded (ds) RNA, single-stranded (ss) RNA or DNA, respectively, that are delivered to endosomes during the infection process. Other receptors are more broadly expressed and almost all cell types can mount an IFN response to cytosolic NA. RIG-I, MDA5 and LGP-2 are helicase proteins that constitute a family of receptors sensing infection with RNA viruses, including influenza A virus and hepatitis C virus, amongst others (54). This cell-autonomous response involves signalling through the adaptor protein IPS-1. Cytosolic DNA triggers IFN induction via an IPS1-independent pathway and DAI has been implicated as a possible receptor (55,56). Other pathways for the recognition of cytosolic NA exist as well. For example, a multi-protein complex termed the inflammasome triggers processing of pro-IL-1 β and pro-IL-18 (57). The mature forms of these cytokines are potent proinflammatory modulators. Recently, a cytoplasmic sensor coupling DNA recognition to the inflammasome has been identified (58–61). Further, viral RNA too has been suggested to trigger the inflammasome (62).

The existence of NA sensors raises an important question of self/non-self discrimination; that is, how do sequence-independent sensors avoid recognition of self-DNA/RNA? Separation of NA from putative receptors, differential modification of endogenous vis-à-vis exogenous NA and disposal of self-NA are all important in this regard (Table 4).

However, such mechanisms are imperfect and it is becoming absolutely clear that the metabolism of endogenous NA is a central theme in the pathogenesis of autoimmunity (Table 5).

Since a hallmark of SLE is the production of antibodies directed against RNA and DNA, the finding of defective NA metabolism in lupus, where NAs can act as both antigen and adjuvant, is unsurprising. Moreover, considering the importance of NA in inducing a type I IFN response, the observation of a disturbance of IFN- α homeostasis as central to the

Table 4. Mechanisms which might be involved in avoiding an immune reaction against self NA

Mechanism	Examples
Physical separation	Endosomal sequestration of TLR3/7/9 (63)
Differentiation by modification	5' triphosphates on viral ssRNAs (64)
'Waste disposal'	DNase I (28)

Table 5. Examples of perturbation of NA metabolism in autoimmune phenotypes

Molecule	Phenotype (reference)
DNase I	Systemic lupus erythematosus (28)
DNase II	Rheumatoid arthritis-like (65)
TREX1 (DNase III)	Systemic lupus erythematosus (10)
FEN1 (DNase IV)	Systemic lupus erythematosus-like (66)
Yaa (TLR7)	Systemic lupus erythematosus (67)
HMGB1/RAGE	Systemic lupus erythematosus (68)
LL37	Psoriasis (69)
P202 (IFI202)	Systemic lupus erythematosus (61)
MDA5 (IFIH1)	Type I diabetes mellitus (70)

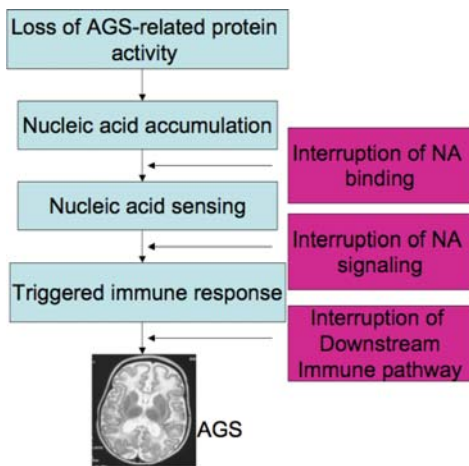


Figure 3. Stages in the pathogenesis of AGS which might be amenable to targeted interruption.

pathogenesis of SLE is also credible. Taken together, these recent studies also offer an elegant mechanistic explanation for the phenotypic overlap of AGS with SLE and congenital infection. That is, in the absence of TREX1, RNASEH2 or SAMHD1 activity, endogenous NAs accumulate and are sensed as 'non-self', leading to the induction of an IFN- α -mediated immune response.

SUMMARY

Broadly speaking, two clinical presentations of AGS can be delineated; an early-onset neonatal form highly reminiscent of congenital infection seen particularly with TREX1 mutations, and a later-onset presentation, sometimes occurring after several months of normal development and occasionally associated with remarkably preserved neurological function,

most frequently due to RNASEH2B mutations (14). Interestingly, whichever presentation, little disease progression seems to occur beyond the initial encephalopathic period. These observations are important because they indicate that the treatment in the early stages of the disease should result in attenuation of the associated inflammation and consequent tissue damage. By defining the precise pathways linking NA accumulation to activation of the immune response we believe that it will be possible to develop treatments, for example antagonists of IRF3 or anti-IFN antibodies, to interrupt the AGS disease process at one or more points (Fig. 3). It is expected that these therapies will be relevant to the treatment of FCL and subtypes of lupus. Exciting precedents exist for such an approach in other immune-mediated inflammatory diseases (71).

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Conflict of Interest statement. None declared.

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