Novel insights into double-stranded RNA-mediated immunopathology

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Abstract

Recent progress in human and mouse genetics has transformed our understanding of the molecular mechanisms by which recognition of self double-stranded RNA (self-dsRNA) causes immunopathology. Novel mouse models recapitulate loss-of-function mutations in the RNA editing enzyme ADAR1 that are found in patients with Aicardi-Goutières syndrome (AGS) — a monogenic inflammatory disease associated with increased levels of type I interferon. Extensive analyses of the genotype–phenotype relationships in these mice have now firmly established a causal relationship between increased intracellular concentrations of endogenous immunostimulatory dsRNA and type I interferon-driven immunopathology. Activation of the dsRNA-specific immune sensor MDA5 perpetuates the overproduction of type I interferons, and chronic engagement of the interferon-inducible innate immune receptors PKR and ZBP1 by dsRNA drives immunopathology by activating an integrated stress response or by inducing excessive cell death. Biochemical and genetic data support a role for the p150 isoform of ADAR1 in the cytosol in suppressing the spontaneous, pathological response to self-dsRNA.

[H1] Introduction

Double-stranded RNA (dsRNA) products are generated during the replication cycle of DNA and RNA viruses[1,2]. Mammalian cells express multiple innate immune receptors, including melanoma differentiation-associated protein 5 (MDA5), protein kinase R (PKR) and Z-DNA binding protein 1 (ZBP1), that bind to and are activated by dsRNA. Each dsRNA receptor activates distinct signalling pathways tailored to restrict virus infection, such as the secretion of antiviral cytokines including type I and type III interferons (IFN-I and IFN-III), the inhibition of translation and the induction of cell death[3]. This antiviral immune reaction is referred to as the dsRNA response (**Box 1**). It is now clear that dsRNA can originate not only from viral nucleic acids but also from endogenous RNA. The first clinical suggestion that impaired dsRNA

metabolism may be causal to human immunopathology was provided by the identification of loss-of-function mutations in *ADAR*, which encodes the dsRNA-specific editing enzyme adenosine deaminase acting on dsRNA 1 (ADAR1), in patients with Aicardi-Goutières syndrome **[G]** (AGS)[4]. We now know that duplex RNA structures generated by base paring of complementary sequences from nuclear or mitochondrial transcripts are potentially rich sources of immunostimulatory dsRNA[5], and that defects in the removal of endogenous RNA duplexes result in the development of a chronic dsRNA response and give rise to human autoinflammatory diseases[5,6]. Here, we extensively discuss how the study of *Adar* loss-of-function mouse models has produced novel insights into the mechanisms of dsRNA-induced immunopathology. We also provide an update on the identity of the endogenous dsRNA species and the dsRNA sensors that underlie autoinflammation in mice and possibly also in humans.

[H1] ADAR1 mutations in AGS

AGS is the prototypical member of a group of monogenic autoinflammatory diseases termed type I interferonopathies[7,8]. All patients with AGS develop neuroinflammatory symptoms and have an increased interferon-stimulated gene (ISG) signature in their blood and cerebrospinal fluid. In addition to AGS, ADAR1 dysfunction can cause other neurological manifestations including bilateral striatal necrosis and progressive spastic paraplegia, which share symptoms with AGS[9-12]. Heterozygous *ADAR* mutations are involved in dyschromatosis symmetrica hereditaria (DSH), a dominantly inherited skin pigmentation disorder with variable penetrance and less pronounced ISG scores than in AGS[4,13,14].

ADAR1 is part of a family of three proteins containing dsRNA-binding motifs (dsRBMs) that allow them to bind to and specifically modify dsRNA[15-20] (**Box 2**). ADAR1 catalyses the hydrolytic removal of an amine group from the C6 position of an adenosine (A), yielding a deaminated inosine (I) nucleobase[21,22]. This process, known as A-to-I editing, generates A:I mismatches that destabilize the dsRNA helix. Human and mouse *ADAR* messenger RNAs (mRNAs) are transcribed from three alternative promotors: two constitutive promotors upstream of exon 1B and exon 1C that give rise to a ~110 kDa protein, and an interferon-inducible promotor upstream of exon 1A that gives rise to a ~150 kDa protein[23-25] (**Fig. 1a**). It should be noted that the smaller p110 isoform can be translated from p150-encoding mRNA through ribosome skipping of the p150 start codon in exon 1A[26,27]. As a result, a fraction of ADAR1-p110 is also interferon inducible. Both p110 and p150 contain a Zβ domain followed by three dsRBMs, which bind to dsRNA with little or no sequence specificity[28,29], and an A-to-

I editase domain (**Fig. 1a,b**). The larger p150 isoform contains an additional amino-terminal Z-nucleic acid **[G]**-binding Zα domain (**Fig. 1a-c**), which also harbours a nuclear export signal (NES)[30,31]. The NES enables the p150 isoform to shuttle between the cytosol and the nucleus, whereas the p110 protein is contained within the nucleus[32]. Human and mouse genetics show that the Zα domain controls spontaneous activation of the dsRNA response (see below), whereas the structurally related Zβ domain is not able to bind to Z-nucleic acids. The Zβ domain may be implicated in protein–protein interactions, although its function remains largely unknown despite its evolutionarily highly conserved sequence[33-35].

AGS-associated *ADAR* mutations can be divided into four categories (**Supplementary Table 1**). The first category comprises homozygous missense mutations in the A-to-I editase domain that (are predicted to) negatively affect the editing activity of ADAR1 (**Fig. 1d**). These mutations likely do not affect the function of the Zα domain and dsRBMs, implying that reduced catalytic activity of ADAR1 underlies autoinflammation. The causal relationship between mutations in the catalytic domain of ADAR1 and autoinflammation was confirmed by the generation of $Adar^{K948N}$ or $Adar^{D963H}$ knock-in mouse models, which recapitulate the human K999N or D1113H homozygous mutations in the A-to-I editase domain[36-38]. The second category comprises heterozygous dominant-negative mutation of glycine at position 1007 of ADAR1 (G1007R). Mapping G1007 onto the available crystal structure of the A-to-I editase domain of the related protein ADAR2 bound to dsRNA shows that this glycine is part of the base-flipping loop of the A-to-I editase domain and lies adjacent to a glutamate that inserts into the dsRNA helix and replaces the adenosine that is to be edited, which is flipped out of the helix into the catalytic pocket of ADAR2 (refs.[39,40]). Replacing glycine 1007 with a bulky arginine residue is predicted to sterically hinder the base-flipping process (**Fig. 1e**). The dominant inheritance of the G1007R mutant implies that it exerts an inhibitory effect on wild-type ADAR1, which may be related to the fact that ADARs typically function as homodimers[41]. Biochemical data show that ADAR2 deficient for dsRNA binding is a dominant-negative inhibitor of A-to-I editing[42]. Whether the G1007R mutation similarly affects the function of ADAR1 homodimers awaits biochemical proof. At least in cellular assays measuring the A-to-I editing efficiency of a known editing substrate, the G1007R mutant has dominant-negative activity over wild-type ADAR1 (refs.[4,43]). A third category of AGS mutations comprises compound heterozygous mutations of ADAR1 combining an editing impaired or null allele of *ADAR* with a Zα-domain-mutant allele (**Fig. 1c**). This allelic combination is seen in more than half of patients with AGS who have *ADAR* mutations and is discussed in detail below. The

fourth category comprises miscellaneous genotypes including homozygous missense mutations of the first dsRBM of ADAR1 and compound heterozygous mutations generating an A-to-I editing-mutant allele combined with an *ADAR* or *ADAR-p150* null allele. Complete ADAR1 deficiency has not yet been reported and is likely incompatible with life; full *Adar*-knockout mice die around embryonic day (E) 11.5–12.5 (refs.[44,45]).

[H1] A-to-I editing prevents immunopathology

In all cases of AGS resulting from *ADAR* mutations the editing activity of ADAR1 is predicted to be reduced, either directly though mutation of the A-to-I editase domain or indirectly by affecting substrate binding through mutation of the Zα domain or first dsRBM or a reduction in gene dosage. Together, these data show that A-to-I editing activity must be maintained above a crucial threshold to restrict dsRNA-mediated immunopathology.

[H2] Substrates of ADAR1

The vast majority of ADAR1-mediated A-to-I editing takes place on RNA duplexes formed by intramolecular base pairing of two inversely oriented repetitive elements[18]. In humans, A-to-I editing occurs almost exclusively on inverted repeat *Alu* (IR-*Alu*) elements[46-50]. *Alu* sequences are around 280 base pairs (bp) in length and are part of the family of short interspersed nuclear elements **[G]** (SINEs). They are enriched in introns and in the 3′ untranslated regions (UTRs) of (pre-)mRNA transcripts, and the approximately 1.2 million *Alu* copies occupy ~13% of the human genome[51]. The interaction of ADAR1 with human *Alu* sequences was confirmed by UV crosslinking and immunoprecipitation followed by RNA sequencing[52]. By contrast, the enormous *Alu* expansion as observed in primates has not occurred in rodents, and in mouse cells, A-to-I editing mainly takes place on B1 and B2 SINEs, and to a lesser extent on long interspersed nuclear elements (LINEs) and long terminal repeats (LTRs)[53,54]. Editing in mice occurs at a lower frequency (by at least 100-fold) than in humans[53,55]. This difference may be attributed to the greater sequence diversity and shorter length of mouse repetitive elements compared with human *Alu* sequences, which reduces the intrinsic stability of potential ADAR1 substrates formed by two inverted repeats in mouse transcripts[53].

It is generally assumed that ADAR1 reduces the intracellular concentration of potentially immunostimulatory dsRNA by destabilizing the dsRNA helix. However, ADAR1 favours editing of adenosine paired with cytosine (A:C mismatch) over adenosine paired with uracil[56,57], which creates more stable I:C base pairs and promotes RNA duplex formation. Parallel analysis

of RNA secondary structure sequencing (PARS-seq) confirmed that the presence of ADAR1 increased global dsRNA structures[58]. How then does ADAR1 prevent the accumulation of immunostimulatory dsRNA? A recent study shows that ADAR1 dimers simultaneously edit adenosines located on both strands of the RNA duplex that are positioned 35 bp upstream and 30 bp downstream from minor helix perturbations[59]. The initial introduction of an I:U mismatch by editing of an A:U pair thus promotes recursive editing at a second site until the dsRNA helix is resolved and can no longer activate dsRNA sensors. Thus, although preferred editing of A:C mismatches may increase the stability of base pairing at some sites, it also negatively influences the recursive editing process. Instead, recursive editing of A:U pairs in longer dsRNA substrates may destabilise immunostimulatory dsRNA. Indeed, long dsRNA helices formed by IR-*Alu* elements or *cis*-natural antisense transcripts **[G]** (*cis*-NATs) that function as endogenous MDA5 agonists (see below) are extensively edited by ADAR1. Alternative and non-mutually exclusive explanations for the non-immunogenic nature of ADAR1 products may include that they have increased sensitivity to inosine-specific nucleases[60-62] and that I:U-containing dsRNA mediates direct immunosuppression[63].

[H2] ADAR1 blocks MDA5 activation

An important step towards understanding the function of ADAR1 was the demonstration that ADAR1 suppresses spontaneous IFN-I signalling[64]. Microarray profiling of fetal liver haematopoietic stem cells from *Adar*-knockout mouse embryos showed that the expression of ISGs was greatly increased, some by more than two orders of magnitude[64]. *Adar*-knockout mice crossed into an MDA5-deficient background or a background deficient for the MDA5 adaptor protein mitochondrial antiviral-signalling protein (MAVS) did not develop a spontaneous IFN-I response and their lethality at embryonic (E) day E12.5 was rescued at least until birth[43,65], showing that MDA5–MAVS signalling is responsible for the aberrant IFN-I phenotype and developmental lethality (**Supplementary Table 2**). The crucial role of ADAR1 in suppressing MDA5 activation was later substantiated in knock-in mouse models corresponding to human *ADAR* mutations found in patients with AGS or conditional knockout models wherein deletion of MDA5 or MAVS rescued the tissue-specific or cell-specific pathological phenotypes (**Supplementary Tables 2 and 3**). Notably, depletion of ADAR1 expression in adult mice resulted in lethal MDA5-dependent and MAVS-dependent autoinflammation[65,66], which indicates that ADAR1-mediated immunosuppression is not only required during development but remains important throughout life (**Supplementary Table 3**).

The cytosolic dsRNA sensor MDA5 cooperatively binds along the axis of a dsRNA helix, forming a dynamic filamentous structure containing multiple activated MDA5 molecules[67,68]. MDA5 then binds to MAVS, which further oligomerises at the mitochondrial outer membrane[69]. This activates a complex downstream signalling pathway involving multiple TRAF family ubiquitin ligases and the TBK1–IKKε and IKKα–IKKβ kinases, which activate the transcription factors IRF3, IRF7 and nuclear factor-κB (NF-κB) to induce expression of IFN-I and IFN-III (**Fig. 2**), ISGs and proinflammatory cytokines[70-72]. In addition to inducing a transcriptional response, MDA5–MAVS activation has also been reported to trigger apoptosis directly, although the molecular mechanism remains unclear[73-75].

Surprisingly, crossing *Adar*-knockout mice into an IFN-I signalling-deficient background by deleting the IFN-I receptor subunit IFNAR1 only delayed embryonic lethality by four days[43] (**Supplementary Table 2**). Combined ablation of IFNAR1 and the IFNγ receptor subunit IFNGR1 did not further increase the survival of *Adar*-knockout embryos[76], which suggests that IFN-γ (IFN-II) signalling is not involved in pathology. IFN-III, which induces a largely overlapping set of genes to IFN-I[77], could also contribute to embryonic lethality. However, genetic removal of the STAT1 signal transducer and transcription factor, which functions downstream of receptors for IFN-I, IFN-II and IFN-III[78], did not further rescue the lethality of *Adar*-knockout embryos[43]. This shows that interferon signalling does not greatly contribute to the embryonically lethal phenotype caused by complete loss of ADAR1 function. IRF3, which is activated immediately downstream of MAVS, and the ISGF3 transcription factor complex (containing STAT1, STAT2 and IRF9), which is activated downstream of the IFN-I or IFN-III receptor, control the expression of many of the same genes[79]. Thus, cell-intrinsic MAVS signalling may suffice to induce pathology without the need for interferon-mediated positive feedback, at least in the context of complete loss of ADAR1 (**Fig. 3**). This is supported by the observation that blocking signalling induced by IFN-I[43] or both IFN-I and IFN-γ[76] did not fully prevent ISG expression in *Adar*-knockout mice. Interestingly, however, genetic removal of either IRF3 in *Adar*-knockout mice[80] or of IRF7 in *Adar* editing-deficient mice[81] also fails to rescue embryonic lethality. *Adar/Irf3* double-knockout embryos still developed an ISG signature[80]. Since loss of IRF3 can be compensated by IRF7 (ref.[82]),deletion of both IRF3 and IRF7 is most likely required to fully prevent ISG expression downstream of MDA5–MAVS and to recapitulate the rescue of *Adar*-knockout embryos seen by removal of MDA5 or MAVS, although this remains to be addressed experimentally. It is also possible that interferon-

independent mechanisms, including NF-κB overactivation and/or uncontrolled apoptosis, contribute to the MAVS-mediated developmental defects.

As we discuss later, blockade of IFN-I signalling is sufficient to prevent autoinflammatory pathology in 'milder' *Adar* genotypes, such as hemizygous *Adar* Zα domain-mutant mice[83], which suggests that the need for IFN-I-mediated amplification of the autoinflammatory immune response may depend on the severity of the *Adar* genotype and the magnitude of the spontaneous dsRNA response elicited in these mice.

[H2] Editing-(in)dependent functions of ADAR1

Conclusive evidence in favour of an immunosuppressive role for the enzymatic activity of ADAR1 came from the generation of ADAR1 editing-deficient mice[84]. Replacing glutamine 861 of ADAR1, an active site residue that functions as a proton shuttle during the deamination reaction[85], with an alanine (E861A) completely abolishes A-to-I editing activity[86]. Mice expressing E861A-mutant ADAR1 from both alleles phenocopy the embryonic lethality of complete *Adar*-knockout mice and similarly develop a spontaneous MDA5-mediated IFN-I response[84]. This demonstrates that A-to-I editing is the primary mechanism by which ADAR1 prevents immunopathology. Accordingly, homozygous missense mutations in the A-to-I editase domain cause AGS in humans (**Supplementary Table 1**). It should be noted that ADAR1 editing-deficient mouse embryos survive one embryonic day longer than full *Adar*-knockout mice[44,45,84], which suggests that ADAR1 also has editing-independent functions. This small difference in embryonic viability becomes remarkably apparent when these animals are crossed into an MDA5-deficient background. Whereas ADAR1 E816A-mutant, MDA5-deficient animals continue to thrive well into adulthood and are largely indistinguishable from their MDA5-deficient littermates[66], full *Adar/Mda5* double-knockout mice are runted and die before weaning[65] (**Supplementary Table 2**). At least two non-mutually exclusive mechanisms might underlie these phenotypic differences. First, catalytically dead ADAR1 can still bind dsRNA and may sequester it from MDA5, which could briefly extend the lifespan of editing-deficient embryos. Second, and as discussed in detail below, sequestration of dsRNA by editing-deficient ADAR1 may further inhibit downstream activation of the IFN-inducible nucleic acid sensors ZBP1 and PKR. Thus, although A-to-I editing has the major role in preventing spontaneous MDA5 activation, editing-deficient ADAR1 may still function as a buffer to prevent spontaneous activation of other dsRNA sensors.

[H2] MDA5 agonists

The fact that ADAR1 mainly edits IR-*Alu* sequences suggested that these dsRNA structures could be MDA5 agonists. Indeed, an RNase protection assay identified IR-*Alu* sequences within 3′ UTRs of mRNAs as being the main RNA species associated with active MDA5 filaments[87] (**Fig. 2b**). The highly abundant intronic IR-*Alu* sequences likely pose no danger of being detected by MDA5 as they are removed by the splicing machinery before mRNAs enter the cytoplasm where MDA5 is located. In fact, IR-*Alu* sequences within 3′ UTRs, but not those within introns, are subject to strong purifying selection, likely providing a first line of defence against unintended activation of MDA5 (ref.[88]). ADAR1 may then function as a second barrier to preventing spontaneous MDA5 activation by IR-*Alu* sequences within 3′ UTRs that have escaped purifying selection. Interestingly, gain-of-function mutants of MDA5 identified in patients with AGS[89,90] are activated more efficiently by IR-*Alu* sequences, even in their edited forms[87]. This shows that either lowering the activation threshold of MDA5 through gain of function or increasing the cytosolic availability of immunostimulatory dsRNA through ADAR1 loss of function can underlie inflammatory disease development.

A recent study correlating quantitative trait loci that are associated with reduced A-to-I editing with genetic variants that are associated with common inflammatory diseases proposed that dsRNA formed by intermolecular base pairing of *cis*-NATs constitutes a second source of MDA5 agonists[91] (**Fig. 2b**). *Cis*-NATs are transcribed from opposing DNA strands of the same genomic locus[92]. As a result, they can form perfect RNA duplexes and are subject to A-to-I editing[56,91]. Sequence erosion among individual *Alu* elements determines that even in their unedited form IR-*Alu* sequences contain on average 48 mismatches spanning a 238 bp dsRNA[91]. Their relatively short length and the abundance of mismatches and bulges render IR-*Alu* sequences inherently poor MDA5 agonists[87]. By contrast, the perfectly base-paired *cis*-NATs, which measure on average 611 bp, better meet the biochemical requirements for strong MDA5 activation, which requires perfect RNA duplexes of >500 bp in length[93]. However, IR-*Alu* sequences are vastly more abundant than *cis*-NATs and the odds of forming RNA duplexes may be more favourable for IR-*Alu* sequences than for *cis*-NATs as it requires intramolecular base pairing rather than hybridisation of two separate transcripts. Sensing of shorter dsRNA such as those formed by IR-*Alu* sequences may be aided by co-factors such as the MDA5 paralogue laboratory of genetics and physiology 2 (LGP2), which promotes the cooperative assembly and activation of MDA5 onto shorter stretches of dsRNA[94]. Indeed, LGP2 is crucial for inducing an MDA5-mediated IFN-I response in ADAR1 Zα domain-mutant mice[83] (see

below) and ADAR1-deficient human cells[95] (**Fig. 2b**). The relative contributions of IR-*Alu* sequences and *cis*-NATs to immunopathology remain to be determined.

[H1] ADAR1-p150 prevents immunopathology

Genetic analyses show that some patients with AGS carry a Zα domain-mutant or A-to-I editase domain-mutant *ADAR* allele combined with a second allele containing a point mutation that abrogates the ADAR1-p150-specific start codon generating a *p150*-null allele[96,97] (**Supplementary Table 1**). As these mutations are expected to leave ADAR1-p110 expression unaffected, these data provide compelling clinical evidence that the p150 isoform is crucial for suppressing immunopathology in humans.

[H2] The ADAR1-p150 negative-feedback loop

The role of ADAR1-p150 in suppressing a lethal dsRNA response was experimentally demonstrated by generating mice in which exon 1A of *Adar* was disrupted, resulting in loss of p150 expression while retaining constitutive p110 expression[98]. These mice die at E12.5 and develop a spontaneous IFN-I response, thereby largely phenocopying full *Adar*-knockout mice[98]. Removal of MDA5–MAVS signalling had a greater impact on the survival of ADAR1-p150-deficient mice than of full *Adar*-knockout mice, revealing crucial functions of ADAR1-p110 in kidney and brain development[65]. A caveat of this p150-knockout strategy is that expression of the interferon-inducible p110 isoform, generated by leaky ribosomal scanning of the p150 transcript, is also abrogated. This point was recently addressed by the generation of a novel *Adar-p150*-knockout mouse in which a premature stop codon was inserted in the Zα domain-encoding region of the p150 transcript, resulting in complete loss of ADAR1-p150 expression while retaining interferon-inducible ADAR1-p110 expression[27]. Embryos derived from this *Adar-p150*-knockout line also succumbed at E12.5 (ref.[27]) but a recent preprint shows that the mice survive post-birth when MDA5 is removed[99], which largely reproduces previous findings on the essential role of ADAR1-p150 in inhibiting the dsRNA response[65]. The non-redundant immunosuppressive role of ADAR1-p150 was further substantiated by the generation of *Adar-p110*-specific knockout mice[100]. Most of these mice still died after birth, a phenotype which was not rescued by MDA5 deficiency[100]. Mice that do not express ADAR1-p110 do not develop a spontaneous IFN-I response[100], demonstrating that ADAR1-p150 and not the p110 isoform prevents spontaneous MDA5 activation and that the p110 isoform regulates vital MDA5-independent processes such as those required for normal kidney and brain function[65].

Although ADAR1-p150 and ADAR1-p110 share a nuclear localisation signal[101,102], p150 contains an additional nuclear export signal that enables its nucleocytosolic shuttling[30,31] (**Fig. 1a**). The non-redundant immunosuppressive role of ADAR1-p150 can most likely be attributed to its cytosolic localisation, where its main task would be to destabilise potentially immunostimulatory dsRNA molecules. Indeed, cytosolically overexpressed ADAR1-p110 can attend to the same substrates as the p150 isoform[103] and two preprint articles show that forced cytosolic expression of ADAR2 suppresses MDA5-mediated ISG expression[104] or PKR activation[99], which suggests that cytosolic localisation is an important determinant of substrate specificity and immunosuppression, at least in overexpression settings. Another factor determining the unique immunosuppressive effect of ADAR1-p150 is the fact that its presence in the cytosol is rapidly induced upon interferon stimulation. Inflammation was previously shown to enhance A-to-I editing activity *in vivo*[105], and this activity was later shown to depend solely on increased ADAR1-p150 expression in mouse[106] and human cells[107]. Of note, the expression of potentially immunostimulatory endogenous dsRNA is enhanced by IFN-I signalling (discussed later)[108] (**Fig. 2a**). In addition, MDA5 and other immune sensors including PKR and ZBP1 (see below) are also ISGs. The activation threshold of these sensors depends not only on the availability of their activating dsRNA ligands but also on the expression levels of the sensor itself[5]. We therefore propose a model in which the interferon-inducible expression of ADAR1-p150 in the cytosol acts as a negative-feedback mechanism to deal with the increased responsivity to self-dsRNA during inflammation owing to increased expression of dsRNA sensors and their potential agonists (**Fig. 2c**). Only when the increased concentration of immunostimulatory dsRNA and increased expression of dsRNA sensors cannot be matched by enhanced ADAR1-p150-mediated A-to-I editing and sequestration of dsRNA will the activation threshold of dsRNA sensors be reached and a breach in tolerance against self-dsRNA occur.

[H2] ADAR1 and Z-nucleic acids

In addition to its cytosolic localisation and interferon inducibility, another unique feature of ADAR1-p150 is the presence of a Zα domain (**Fig. 1a**). Zα domains bind specifically to left-handed Z-nucleic acids, including Z-RNA[109,110] (**Box 3**). The relevance of the Zα domain to human health became apparent from the observation that more than half of patients with AGS who have *ADAR* mutations carry compound heterozygous mutations whereby one *ADAR* null or editing-impaired allele is combined with a proline 193 to alanine (P193A) Zα domain-mutant

allele[4,12]. Other compound heterozygous Zα domain mutations including P193L[111] and N173S[9] (**Fig. 1c** and **Supplementary Table 1**) have also been identified in patients with AGS or bilateral striatal necrosis, which further supports the importance of the Zα domain in preventing disease. Asparagine 173 is part of the α3 recognition helix of the Zα domain and forms a hydrogen bond with Z-nucleic acids whereas proline 193 forms a van der Waals contact with the sugar-phosphate backbone of Z-DNA[112] and Z-RNA[113]. Mutations of these residues to alanines decrease the affinity for Z-DNA[114], suggesting that binding of ADAR1 to Z-nucleic acids is crucial for its immunosuppressive function. To test this hypothesis, several groups generated Zα domain-mutant mice with the intention to disrupt the interaction of ADAR1 with Z-nucleic acids[83,115-120]. Three groups reported P195A Zα domain-mutant mice orthologous to the AGS-associated P193A mutation[83,119,120] and four other groups described N175A and Y179A[115,116] (N175A+Y179A), N175D+Y179A[118] mutation (corresponding to N173 and Y177 in human ADAR1) or W197A[117] mutations (corresponding to W195 in human ADAR1). Based on structural assays[112,113] and biochemical Z-DNA binding assays[114] using human ADAR1, these mutations are expected to perturb the Zα domain function as follows, in order of least disruptive to most disruptive: P193A < Y177A+N173A <<< W195A. Proline 193 in human ADAR1 is located in the wing of the Zα domain β hairpin (**Fig. 1c**) and its mutation to alanine decreases its affinity for Z-DNA, whereas combined N173A+Y177A mutation within the α3 recognition helix completely prevents binding to Z-DNA[114] (**Fig. 1c**). Although N173A+Y177A mutation does not disrupt domain folding[121], the W195A mutation within the β3 sheet is predicted to have a marked impact on Zα domain structure, resulting in loss of domain architecture[112-114]. The biochemical consequences of these mutations are reflected with remarkable accuracy by the in vivo impact of introducing the corresponding mutations in mice. Mouse W197A (human W195A) in the homozygous state is lethal post-birth and these mice develop a strong MDA5-dependent IFN-I response[117]. By contrast, homozygosity of the mouse N175A+Y179A[115,116], or N175D+Y179A[118] mutations (human N173A/D + Y177A) or P195A mutation[37,83,119,120] (human P193A) is well-tolerated, resulting in only slightly increased ISG expression across various tissues[83,115,116,118,120]. However, combining the mouse N175A/D+Y179A or N175D+Y179A alleles with an *Adar*-null allele, resulting in hemizygous Zα domain-mutant ADAR1 expression and thereby recapitulating the genetics of patients with AGS, causes immediate postnatal mortality accompanied by a strong MDA5–MAVS-induced ISG signature[115,118,122]. The phenotype of combining the less disruptive mouse P195A allele with a p150-specific[83] or full[119,120] *Adar*-knockout allele is milder, inducing later onset of lethal pathology[83], and with some mice of a more recent lines even showing normal survival[119,120].

Differences in genetic background or animal housing may explain these discrepancies. We anticipate that less penetrant *Adar*-mutant alleles such as the mouse P195A variant may be particularly susceptible to minor genetic or environmental differences that set the threshold for disease initiation.

Early cellular and biochemical assays on a limited number of editing substrates showed that the Zα domain of ADAR1-p150 promotes A-to-I editing[43,123]. Recent global RNA-sequencing-based profiling of A-to-I editing shows that Zα domain mutations induce rather subtle changes in global A-to-I editing at steady state[115-120]. Some A-to-I sites even have increased editing when binding of ADAR1 to Z-nucleic acids is abrogated[115-119], which most likely reflects the increased interferon-induced expression of ADAR1-p150 in Zα domain-mutant cells. This results in an apparent net zero effect on the A-to-I editing profile. It is important to note that these A-to-I editing profiles were determined in steady state conditions or in the mildly inflammatory background of homozygous Zα domain mutation. By contrast, when editing profiles within repeat elements are enumerated in conditions wherein the demand for negative feedback by ADAR1-p150 is high, for instance after IFN-I stimulation[122] or in highly inflammatory tissues from hemizygous Zα domain-mutant mice[118], mutation of the Zα domain results in a marked decrease in editing efficiency both at individual sites and across entire repeat elements. This shows that the interaction of ADAR1 with Z-nucleic acids stimulates global A-to-I editing of repeat elements when the demand for editing is increased such as during inflammation. It should be noted that a recent study reported no differences in A-to-I editing levels in the brains of hemizygous *Adar* P195A Zα domain-mutant mice[120]. A possible explanation for this observation, apart from the fact that the Zα domain-containing p150 isoform is not expressed in brain tissue[100,120], is that these analyses were performed in MDA5-deficient mice, which do not develop an IFN-I response and in which the expression of possible immunostimulatory ADAR1-p150 substrates is not enhanced. So far, no Z-nucleic acid-prone sequence motifs have been identified in the vicinity of Zα domain-dependent editing sites[116,117,119]. This is in line with the observation that the sequence specificity of Zα domains may be much broader than initially anticipated[124]. Thus, the interaction of ADAR1 with Z-nucleic acids is crucial to prevent dsRNA-mediated immunopathology. More work is needed, however, to understand the molecular identify of the Z-nucleic acid interaction partners of ADAR1.

[H1] Suppression of PKR and ZBP1 activation

Although genetic blockade of MDA5 signalling rescues the embryonic lethality of *Adar*[43] or *Adar-p150*[65] deficient mice, it does not does not prevent postnatal death, suggesting that dsRNA sensors other than MDA5 may contribute to pathology. Indeed, recent work shows that ADAR1 additionally inhibits pathogenic engagement of the dsRNA receptor PKR and the Z-nucleic acid sensor ZBP1.

[H2] Inhibition of PKR activation

ADAR1-mediated suppression of the dsRNA sensor PKR was first reported in virus-infected cells[125] and was further substantiated in a large number of human and mouse cell lines[20,107]. PKR engagement activates the integrated stress response **[G]** (ISR) by phosphorylating serine 51 on eukaryotic translation initiation factor 2α (eIF2α), which is part of the ternary translation initiation complex. This single phosphorylation blocks GDP-to-GTP exchange by the guanine nucleotide exchange factor eIF2B, resulting in a general reduction in protein synthesis[126]. Stalling of translation initiation by PKR at the same time promotes the translation of a subset of mRNAs including mRNA encoding the transcription factor ATF4, which induces the expression of ISR-associated genes[126]. Expression of these genes has been detected in the kidney and liver of ADAR1 Zα domain-mutant mice, albeit to varying degrees depending on the mouse line[83,99,119,120]. Remarkably, treatment with a drug that relieves the inhibitory effect of eIF2α phosphorylation on translation completely prevented lethal pathology in a hemizygous ADAR1 P195A Zα domain-mutant mouse line with a fully penetrant phenotype[83], providing a potential therapeutic target for patients with AGS who carry this *ADAR* mutation. These mice are also rescued by an IFN-I signalling-deficient background, which has established a model whereby MDA5-mediated IFN-I production enhances PKR activation, which in turn exerts immunopathology by inducing a chronic ISR[83] (**Fig. 2a**).

A recent preprint further reports that deletion of the dsRNA sensor PKR rescues *Adar*/*Mda5* double-knockout mice beyond the early postnatal lethality, with 50% of the animals reaching adulthood[99], which shows that PKR activation can occur independently of MDA5 and does not necessarily require autocrine or paracrine IFN-I stimulation, at least when ADAR1 protein is completely absent. Removal of both PKR and MDA5 from ADAR1-p150-deficient animals completely restores viability, demonstrating that the p150 isoform forms an important physiological brake on pathological PKR activation[99]. In contrast to complete *Adar*/*Mda5* double-knockout mice, ADAR1 editing-deficient mice in an MDA5-deficient background did not develop a pronounced ISR[99], providing *in vivo* evidence that inhibition of PKR is to a large

extent independent of the editing function of ADAR1. This is supported by biochemical data showing that PKR inhibition crucially depends on cytosolic sequestration of self-dsRNA by the three dsRBMs of ADAR1-p150 (refs.[99,107]). However, a contribution of ADAR1 editing function to PKR inhibition should not be excluded, particularly during an IFN-I response when levels of self-dsRNA are increased[108].

[H2] Inhibition of ZBP1 activation

The Z-nucleic acid sensor ZBP1 is the only mammalian protein other than ADAR1 that has a Zα domain (**Box 3**). Deficiency of ZBP1 restores the viability of ~40% of *Adar*/*Mavs* double-knockout mice until at least 15–30 weeks of age[118,122], providing clear genetic evidence that ADAR1 not only represses MDA5 and PKR but also regulates ZBP1 activation. Similarly, the lethal pathology of hemizygous ADAR1 Zα domain-mutant mice was partially rescued by crossing to *Zbp1*-knockout mice[118,127] or mice that encode a Zα domain-mutant ZBP1 protein that cannot interact with Z-nucleic acids[118,122]. Activation of ZBP1 results in the recruitment of the RIPK3 signalling kinase, thereby establishing a signalling complex that enables activation of NF-κB[128-130] and of RIPK1–FADD–caspase-8-dependent apoptotic[131,132], MLKL-mediated necroptotic[131-133] and NLRP3-mediated pyroptotic[132,134,135] cell death pathways. Loss of ADAR1 sensitises mouse and human cells to ZBP1-mediated apoptosis, necroptosis[108,118,122,127,136] and pyroptosis[136] (**Fig. 2a**). In some cell types, activation of ZBP1 as a consequence of ADAR1 deficiency also required inhibition of translation[118,122] or of nuclear export[136], which suggests that inhibitory proteins such as cFLIP[137] may regulate ZBP1-induced cell death. It will be interesting to test whether PKR-mediated translational arrest is a physiological cue that sensitises cells to ZBP1-mediated cell death.

Deletion of RIPK3 in *Adar*/*Mavs* double-knockout mice provides the same protection as ZBP1 deficiency, which is in line with the crucial role of RIPK3 in initiating ZBP1-mediated cell death[118]. Importantly, this suggests that cell death is the main driver of ZBP1-dependent pathology, although a contribution of RIPK3 to NF-κB signalling cannot be excluded. Unexpectedly, RIPK3 deficiency offered little or no survival advantage in mice carrying a Zα domain-mutant allele of *Adar* combined with an *Adar*-null[118,122] or *Adar-p150*-null[127] allele. In line with this, combined deletion of FADD and MLKL, FADD and RIPK3 (ref.[118]), caspase-8 and RIPK3 (ref.[127]), or caspase-8 and MLKL[122], which blocks execution of both apoptosis and necroptosis, did not result in a rescue phenotype[118,122] or worsened pathology, possibly owing to uncontrolled NF-κB-mediated inflammation in the absence of caspase-8 (ref.[127]). It should

be noted that ADAR1 Zα domain-mutant mice still develop a strong IFN-I response, which occurs independently of apoptosis and necroptosis induction[118], whereas ZBP1 deficiency reduces but does not completely prevent ISG expression, as is the case in a *Mavs*-deficient background[118,122,127]. Recent reports show that IFN-I induction downstream of ZBP1 involves MAVS[138] or cooperation with the dsDNA sensor cGAS[139]. The exact molecular mechanism of ZBP1-mediated IFN-I induction and whether this contributes to pathology are important unresolved questions.

ZBP1 and ADAR1 interact with a common substrate through their Zα domains[127,136], which suggests that they might compete for the same molecules. One study suggests that ZBP1–ADAR1 interaction bridged by a common substrate prevents RIPK3 recruitment to ZBP1 (ref.[136]). IFN-I stimulation of ADAR1-deficient cells increases the intracellular concentration of Z-RNA[108]. Z-RNA immunoprecipitation followed by sequencing showed that a large fraction of Z-RNA-containing transcripts are in fact ISGs, which explains the IFN-I-induced increase in Z-RNA concentration[108]. Some of these Z-RNA-forming sequences were identified as short stretches of dsRNA whereas others constituted IR-*Alu* sequences[108]. Transfection of IR-*Alu* sequences in ZBP1-expressing human cell lines induces cell death, which suggests that Z-RNA-forming IR-*Alu* sequences may be ZBP1 agonists[122]. Despite these findings, direct protein–RNA interaction studies complemented with cellular activation assays are needed to ascertain the identity of ZBP1 agonists in the absence of ADAR1 function. Finally, the Zα domain of ADAR1-p150 was shown to be crucial for suppressing Z-RNA accumulation, whereas A-to-I editing had less impact on Z-RNA accrual[108], which suggests that sequestration of Z-RNA is the primary mechanism by which ADAR1 prevents ZBP1 activation.

[H1] Concluding remarks

The study of *Adar* loss-of-function mice now places MDA5–MAVS-induced IFN-I signalling at the centre of dsRNA-induced immunopathology. MDA5 controls the expression of many ISGs, including those encoding PKR and ZBP1, which through the induction of an ISR or regulated cell death cause autoinflammation. Sequestration of self-dsRNA molecules by the Zα domain and dsRBMs of ADAR1 and A-to-I editing of endogenous dsRNA molecules are two important mechanisms by which ADAR1 restricts activation of MDA5, PKR and ZBP1. The suppression of MDA5 activation mainly involves editing-dependent mechanisms, whereas PKR and ZBP1 inhibition mostly depend on sequestration of dsRNA. Under highly inflammatory conditions, however, wherein both dsRNA concentrations and levels of the

immune sensors are strongly increased, both editing-dependent and editing-independent mechanisms are likely to be required to keep all three innate immune receptors below their activation threshold.

It is important to emphasise that the pathogenic effector mechanisms and the relative contributions of MDA5, PKR or ZBP1 to disease vary across the different *Adar* loss-of-function models. Full *Adar*-knockout mice do not equate to *Adar-p150*-specific knockout mice or editing-deficient mice. Similarly, hemizygous ADAR1 Zα domain-mutant mice that recapitulate the compound heterozygous state of patients with AGS can have surprising heterogeneity in their pathological manifestations, disease penetrance and rescue phenotypes. We propose that at least two parameters determine whether a breach in tolerance to self-dsRNA occurs and overt pathology develops (**Fig. 3**). The first parameter is the capacity of ADAR1-p150 to provide negative feedback through A-to-I editing of cytosolic dsRNA and sequestration of dsRNA or Z-RNA. As such, mild MDA5-driven IFN-I signalling in homozygous P195A Zα domain-mutant mice induces sufficiently high levels of ADAR1-p150 to reduce and/or sequester the increased concentrations of immunostimulatory dsRNA without inducing pathology (**Fig. 3b**)[83,119,120]. The second parameter is the requirement of positive feedback through IFN-I signalling. For example, IFN-I signalling contributes very little to the lethality of full *Adar*-knockout mice[43,76] or hemizygous N175A/Y179A Zα domain-mutant mice (our unpublished data), whereas hemizygous P195A Zα domain-mutant mice are fully rescued by IFNAR1 deletion[83]. In the future, it will be important to consider these genotype–phenotype relationships for preclinical experimentation aimed at reducing dsRNA-induced immunopathology.

To enable more precise therapeutic intervention, it will be crucial to further characterise the pathogenic effector mechanisms acting downstream of PKR and ZBP1. In the case of PKR, chemical inhibition of the ISR is a promising therapeutic approach[83]. In the case of ZBP1, more work is needed to understand how genetic inhibition of both apoptosis and necroptosis does not rescue pathology to the same extent as ZBP1 deficiency and how ZBP1 promotes IFN-I induction. Although this Review focuses on the pathogenic roles of MDA5, PKR and ZBP1, it is very likely that other dsRNA-sensing immune pathways — including Dicer, OAS–RNase L, NLRP1 and NLRP6 — may also contribute to dsRNA-mediated pathology. Although ADAR1 has been shown to inhibit RNase L in human cells[140], the OAS–RNase L system does not overtly contribute to the lethality of hemizygous ADAR1 Zα domain-mutant mice[83]. It is

possible that the pathological functions of these alternative dsRNA-sensing systems result in phenotypes that have been overlooked or have a more pronounced effect in human disease. Finally, although the study of *ADAR* loss-of-function represents an extreme case of dsRNA-mediated pathology, it may provide clues to the pathological mechanisms of other diseases that might involve disrupted dsRNA metabolism, such as type 1 diabetes, systemic lupus erythematosus and dystonia[5].

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Author contributions

R.d.R. and J.M. researched and discussed the cited literature. R.d.R. and J.M. wrote the main text. R.d.R. and J.M. generated the figures and supplementary tables.

Competing interests

The authors declare no competing interests.

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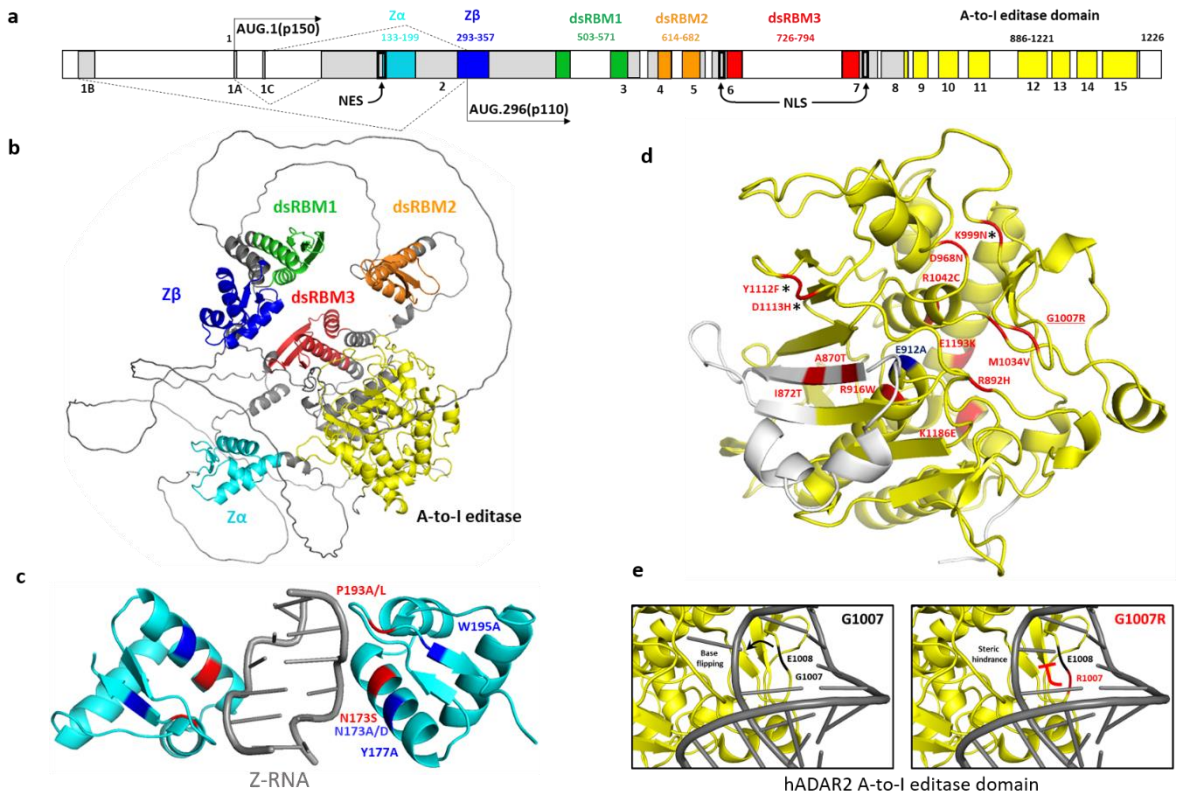

Figure 1 | ADAR1 mutations in Aicardi-Goutières syndrome. a,b, Domain organisation (**a**) and AlphaFold structural prediction (**b**) of human adenosine deaminase acting on dsRNA 1 (ADAR1). Translation of the interferon-inducible ADAR1-p150 isoform starts in exon 1A, whereas translation of the shorter p110 isoform commences at an internal start codon (AUG.296) in exon 2. ADAR1-p150 contains an amino-terminal Zα domain with a nuclear export signal (NES), a Zβ domain, three double-stranded RNA-binding motifs (dsRBM1 to dsRBM3), a bimodular nuclear localisation signal (NLS) and a carboxy-terminal A-to-I editase domain. ADAR1-p110, which is regulated by a constitutive promotor upstream of exon 1B or 1C, lacks the Zα domain and NES. **c**, Crystal structure of human ADAR1-p150 Zα domains binding in an antiparallel manner along the axis of a Z-RNA helix (Protein Data Bank (PDB) code 2GXB)[113]. Mutations in human ADAR1 (P193A/L or N173S) identified in patients with Aicardi-Goutières syndrome (AGS) are indicated in red and the human N173A/D, Y177A or W195A mutations yielding a dysfunctional Zα domain and which have been introduced in *Adar* knock-in mice are indicated in blue. W195 in human ADAR1 (W197 in mouse) connects the carboxy-terminal β-sheet to the α-helical core. N173, Y177 and P193 in human ADAR1 (N175, Y179 and P195A in mouse) mediate direct binding of Z-nucleic acids. **d,** Mutations found in patients with AGS annotated within the predicted Alphafold structure of the ADAR1 A-to-I editase domain. Homozygous missense mutations are indicated with an asterisk; the dominant-

negative G1007R mutation is underlined. Other mutations that are shown have been identified in a compound heterozygous state with a P193A Zα domain-mutant allele or a null allele of *ADAR*. The E912A mutation (in blue), which is equivalent to mouse E861A, completely abrogates the deaminase activity of ADAR1. **e,** Mapping of the G1007R mutation on the crystal structure of ADAR2 (PDB code 5HP2)[39], illustrating the predicted steric hindrance of the bulky arginine (R1007) residue on the base-flipping process by the adjacent glutamate (E1008).

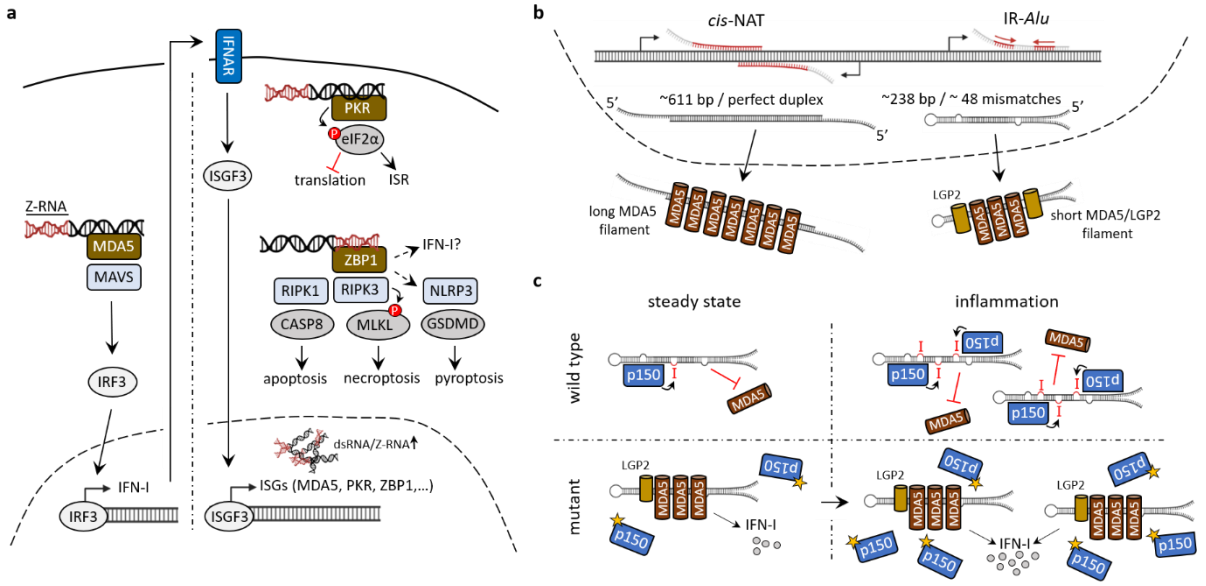

Figure 2 | ADAR1-p150 regulates the double-stranded RNA response. a, In the absence of function of the p150 isoform of adenosine deaminase acting on double-stranded RNA 1 (ADAR1-p150), the innate immune sensor melanoma differentiation-associated protein 5 (MDA5) binds to endogenous double-stranded RNA (dsRNA), which activates the adaptor protein mitochondrial antiviral-signalling protein (MAVS) and induces activation of the transcription factor IRF3, resulting in the expression of type I interferon (IFN-I). Paracrine or autocrine engagement of the IFN-I receptor IFNAR increases expression of the interferon-stimulated genes (ISGs) MDA5, protein kinase R (PKR) and Z-DNA binding protein 1 (ZBP1) and their respective dsRNA and Z-RNA agonists by activating the ISGF3 transcription factor complex. Sensing of self-dsRNA by PKR triggers phosphorylation of eukaryotic translation initiation factor 2α (eIF2α), which leads to general translational arrest and the induction of ATF4-mediated transcription as part of the integrated stress response (ISR). Binding of endogenous Z-RNA to ZBP1 results in RIPK3 recruitment and induction of RIPK1–FADD–caspase-8-dependent apoptosis, MLKL-dependent necroptosis and NLRP3–GSDMD-dependent pyroptosis. **b,** Intermolecular pairing of *cis*-natural antisense transcripts (*cis*-NATs)

or intramolecular hybridisation of inverted repeat Alu (IR-*Alu*) elements generates endogenous MDA5 agonists. Multiple MDA5 proteins, aided by the co-factor laboratory of genetics and physiology 2 (LGP2), cooperatively bind to double-stranded *cis*-NATs or IR-*Alu* sequences, generating filaments containing active MDA5. **c,** At steady state, ADAR1-p150 prevents MDA5 filament formation by adenosine to inosine (A-to-I) editing and sequestration of endogenous dsRNA (upper left panel). IFN-I signalling during inflammation increases MDA5 expression and increases the concentration of endogenous dsRNA. Simultaneous upregulation of ADAR1-p150 leads to an increase in A-to-I editing and sequestration of self-dsRNA to maintain tolerance (upper right panel). Loss of ADAR1-p150 function results in the accumulation of immunostimulatory endogenous dsRNA that triggers MDA5 activation and IFN-I production (lower left panel). This, in turn, results in activation of a MDA5–IFN-I-mediated positive-feedback loop. Increased expression of mutant ADAR1-p150 is unable to inhibit this process, resulting in immunopathology (lower right panel).

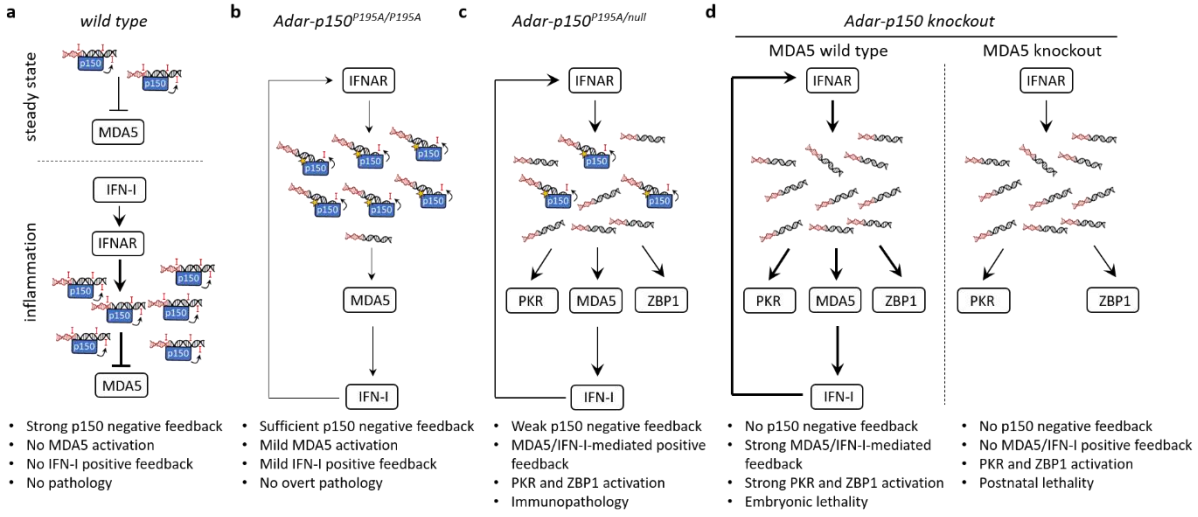

Figure 3 | Negative feedback by ADAR1-p150 and positive feedback through interferon signalling set a threshold for double-stranded RNA-induced autoinflammation. a, Adenosine to inosine (A-to-I) editing and sequestration of endogenous double-stranded RNA (dsRNA) by the p150 isoform of adenosine deaminase acting on dsRNA 1 (ADAR1-p150) prevent spontaneous activation of the dsRNA sensor melanoma differentiation-associated protein 5 (MDA5) during steady state (left). Type I interferon (IFN-I)-induced ADAR1-p150 expression maintains tolerance to increased levels of self-dsRNA during inflammation (right). **b,** Mice expressing P195A Zα domain-mutant ADAR1-p150 from both alleles develop a mild MDA5-induced IFN-I signature. This results in increased expression of Zα domain-mutant ADAR1-p150. Negative feedback through Zα domain mutant ADAR1-p150-mediated editing

and sequestration of dsRNA is sufficient to prevent pathological MDA5-mediated IFN-I signalling. **c,** Hemizygous expression of P195A Zα domain-mutant ADAR1-p150 is unable to edit and sequester enough endogenous dsRNA to suppress MDA5 activation (weak negative feedback), resulting in activation of a pathological MDA5–IFN-I-mediated positive-feedback loop. This increases expression of the innate immune sensors protein kinase R (PKR) and Z-DNA binding protein 1 (ZBP1) and the availability of their respective dsRNA and Z-RNA agonists, causing immunopathology. **d,** The complete absence of negative feedback by loss of ADAR1-p150 expression results in a strong and embryonically lethal MDA5–IFN-I-mediated positive-feedback loop and PKR and ZBP1 activation (top). Increased levels of dsRNA and Z-RNA caused by loss of ADAR1-p150 can also directly activate constitutively expressed PKR and ZBP1 without the need for positive feedback by MDA5-induced IFN-I signalling. Unrestrained activation of ZBP1 and PKR in ADAR1-p150/MDA5 double-deficient mice contributes to postnatal lethality (bottom).

Box 1 | The double-stranded RNA response

The retinoic acid-inducible gene I (RIG-I)-like receptors (RLRs) RIG-I, melanoma differentiation-associated protein 5 (MDA5) and laboratory of genetics and physiology 2 (LGP2) bind to double-stranded RNA (dsRNA) in the cytosol through their helicase and carboxy-terminal domains. RIG-I and MDA5, but not LGP2, contain two caspase activation and recruitment domains (CARDs), which bind to the adaptor protein mitochondrial antiviral-signalling protein (MAVS) to initiate downstream signalling. LGP2 functions as a positive regulator of MDA5 activation by promoting the assembly of MDA5 on dsRNA. RIG-I caps blunt-end dsRNA containing di- or tri-phosphorylated 5' termini[141-145]. MDA5 cooperatively assembles on internal dsRNA structures[67,146]. LGP2 binds to both the ends and the stems of dsRNA[147]. Toll-like receptor 3 (TLR3) scans the endosomal lumen for the presence of dsRNA[148]. The RLRs and TLR3 signal through MAVS or the adaptor protein TRIF (TIR-domain-containing adaptor protein inducing interferon-β (IFNβ)) to induce expression of type I and type III interferons (IFN-I and IFN-III) and proinflammatory cytokines[72,149]. Protein kinase R (PKR) binds to dsRNA through two dsRNA-binding motifs (dsRBMs), resulting in dimerization and kinase activition[150]. PKR phosphorylates the eukaryotic initiation factor 2α, which inhibits cap-dependent translation and mounts an integrated stress response (ISR) by inducing translation of activating transcription factor 4 (ATF4)[126,151]. The oligoadenylate synthases (OAS) contain one or more nucleotidyl transferase domains, which upon binding to dsRNA catalyse the formation of oligoadenylate (2′,5′A_n) second messengers. 2′,5′A_n then

bind to and activate ribonuclease L (RNase L), which non-specifically cleaves host and viral RNA[152]. Z-DNA binding protein 1 (ZBP1) contains two Zα domains (**Box 3**) and three receptor-interacting protein (RIP) homotypic interaction motifs (RHIMs). Binding of Z-RNA or Z-DNA to ZBP1 recruits RIP kinase 1 (RIPK1) and RIPK3 through RHIM–RHIM interactions to induce nuclear factor-κB activation, caspase-8-dependent apoptosis, MLKL-mediated necroptosis and/or NLRP3-mediated pyroptosis[153-155]. Other types of dsRNA sensors that are species specific or tissue restricted include NLRP1 in humans[156] and NLPR6 in the intestine and liver[157,158], which couple sensing of dsRNA to inflammasome activation and, in the case of NLRP6, also IFN-I and IFN-III induction. Stem cells are much less responsive to IFN-I and IFN-III than differentiated cells[159] and they use a splice variant of Dicer to promote antiviral RNA interference[160].

Box 2 | ADAR proteins

The mammalian genome contains three double-stranded RNA (dsRNA)-specific adenosine deaminase (ADAR) genes: *ADAR*, *ADARB1* and *ADARB2*, which encode ADAR1 (ref.[161]), ADAR2 (ref.[162]) and ADAR3 (ref.[163]), respectively[15-20]. ADAR1 and ADAR2 are ubiquitously expressed whereas ADAR3 is exclusively expressed in the brain. ADARs contain two (in the case of ADAR2 and ADAR3) or three (in the case of ADAR1) dsRNA-binding motifs (dsRBMs) followed by an A-to-I editase domain. The A-to-I editase domains of ADAR1 and ADAR2 catalyse the conversion of adenosine (A) to inosine (I) by protonation of the amine group at the C6 position[21,22]. ADAR3 has no A-to-I editing activity and functions as a negative regulator of ADAR1 and ADAR2 activities in the brain[164-166]. ADAR1 also contains a Zβ domain and the ADAR1-p150 isoform has an additional Z-nucleic acid-binding Zα domain. Unlike ADAR1, ADAR2 has no role in immunosuppression. The main function of ADAR2 is to carry out site-specific editing of codons within pre-messenger RNA transcripts[164]. A well-studied example of an editing event exclusively mediated by ADAR2 is the deamination of a specific adenosine within exon 11 of the pre-messenger RNA transcript of *GRIA2*, which causes a CAG to CIG (seen as CGG by the translational machinery) codon transition that results in a glutamine to arginine (Q/R) recoding event at position 607 (refs.[162,167,168]). GRIA2 is a subunit of the tetrameric AMPA glutamate receptor ion channel involved in fast excitatory neurotransmission[169]. In the adult human and mouse brain, *GRIA2* Q/R recoding reaches nearly 100% efficiency[167,170]. Replacing glutamine by a positively charged arginine renders the AMPA receptor ion channels almost impermeable to Ca^{2+} ions, which is thought to protect neurons against glutamine-induced neurotoxicity[171]. The importance of this A-to-I editing event is

demonstrated by the fact that the postnatal lethal phenotype of *Adar2*-knockout mice is rescued by replacing the wild-type (Q/Q) allele of *Gria2* with an edited (R/R) allele[172].

Box 3 | Zα domains and Z-nucleic acids

Zα domains belong to the winged helix-turn-helix motif family, which are found in prokaryotic and eukaryotic DNA-binding proteins[173]. Adenosine deaminase acting on dsRNA 1 (ADAR1) and Z-DNA binding protein 1 (ZBP1) are the only mammalian proteins that contain Zα domains[174,175]. Outside of the mammalian proteome, fish PKZ (an orthologue of PKR)[176], RBP7910 of *Trypanosoma brucei*[177], the poxviral E3 protein[34] and the carp herpesviral ORF112 protein[178] also contain Zα domains that enable either activation of or escape from host cell innate immunity. Their unique fold — consisting of 3 α-helices, 3 β-sheets and 2 loops or 'wings' (W), arranged in a α1–β1–α2–α3–β2–W1–β3–W2 topology — enables Zα domains to interact specifically with Z-nucleic acids. Z-helices form when a purine within an alternating pyrimidine–purine sequence adopts a *syn*-conformation, resulting in a left-handed helix turn[109,179]. Z-nucleic acids exist in chemical equilibrium with their right-handed counterparts; however, the electrostatic repulsion between opposing negatively charged sugar-phosphate backbones, which are in closer contact in Z-helices, renders Z-nucleic acids thermodynamically unstable[109,179]. Both sequence and nucleoside modifications determine the propensity of DNA and RNA helices to transition to the Z-conformation. For example, alternating GC repeats more readily adopt a Z-conformation than AT sequences, and cytidine methylation promotes Z-DNA formation[180]. The processes that stabilise Z-nucleic acids inside living cells remain poorly characterised. Zα domains bind in a symmetrical manner along the axis of Z-DNA and Z-RNA helices with a ~5 bp footprint[112-114]. The interaction interface is mediated by electrostatic interactions between the α3 recognition helix and the β2–W1–β3 hairpin of the Zα domain, containing proline 193 in human ADAR1 which is recurrently mutated in patients with Aicardi-Goutières syndrome, with the sugar-phosphate backbone of Z-nucleic acids[112,113] (**Fig. 1c**). Tyrosine 177 of the human ADAR1 Zα domain (and matching tyrosines in other Zα domain-containing proteins) forms the only direct bond with the C8 carbon of the *syn*-form guanosine in the fourth position of Z-RNA and Z-DNA[112,113].

Glossary

Aicardi-Goutières syndrome. Severe autoinflammatory childhood onset encephalopathy, resulting from the activation of the nucleic acid receptors MDA5 or cGAS by endogenous

nucleic acids due to mutations in one of the following genes involved in nucleic acid sensing or metabolism: *ADAR*, *IFIH1*, *TREX1*, *SAMHD1*, *RNASEH2A*, *RNASEH2B*, *RNASEH2C*, *LSM11*, or *RNU7-1*.

Z-nucleic acid. Left-handed double-stranded (ds)DNA, dsRNA or hybrid DNA:RNA structures, characterised by a zig-zag-shaped (hence the name "Z") phosphodiester backbone containing purine-pyrimidine dinucleotide repeat sequences, which adopt alternating syn- and anti-nucleobase conformations.

short interspersed nuclear elements. SINEs; a subclass of short (< 1000 bp), interspersed (non-tandem), non-autonomous retrotransposon-type repeat elements, containing sequences derived from RNA polymerase III-dependent transcripts including 7SL RNA, tRNA and 5S rRNA.

cis-natural antisense transcripts. RNA molecules transcribed from (partially) overlapping sequences on opposing DNA strands within the same genomic locus containing regions of perfect complementarity, enabling the formation of dsRNA helices.

integrated stress response. Evolutionary conserved cellular stress response induced by the eIF2α kinases, HRI, PKR, PERK or GCN2, resulting into a global translational shutdown while increasing ATF4-dependent gene expression.

Table of Contents

This review discusses how the study of novel mouse models of human ADAR1 deficiency has led to the identification of the innate immune receptors recognising endogenous immunostimulatory dsRNA and their respective downstream signalling pathways that induce autoinflammatory pathology.

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Supplementary Table 1 | *ADAR* loss-of-function mutations identified in patients with Aicardi-Goutières Syndrome (AGS)

Category	Classification	Allele 1		Allele 2		Ref.
		cDNA	Protein	cDNA	Protein	
I	Homozygous missense mutations in the A-to-I editase domain	c.2997G>T	p.K999N	c.2997G>T	p.K999N	[1]
		c.3337G>C	p.D1113H	c.3337G>C	p.D1113H	
		c.3335A>T	p.Y1112F	c.3335A>T	p.Y1112F	
II	Heterozygous dominant negative mutation in the A-to-I editase domain	c.3019G>A	p.G1007R	Not affected		[1]
III	**Compound heterozygous mutations combining an impaired or null allele with a Zα domain mutant allele**					
	Missense mutations affecting the A-to-I editase domain paired with a Zα domain P193A mutation	c2608G>A	p.A870T	c.577C>G	p.P193A	[1]
		c2615T>C	p.I872T			
		c2675G>A	p.R892H			
		c.2746C>T	p.R916W			[2]
		c.2902G>A	p. D968N			
		c3100A>G	p.M1034V			
		c3556A>G	p.K1186E			
	Missense mutation in the ADAR1-p150 start codon paired with a Zα domain P193A mutation	c.1A>G	p.M1?			[3]
	Nonsense mutations introducing a premature stop codon paired with a Zα domain P193A mutation	c.1630C>T	p.R544X			[2]
		c.556C>T	p.Q186X			
		c.982C>T	p.R328X			
		c.1314C>A	p.Y438X			[4]
	Frameshift mutations paired with a Zα domain P193A or N173S mutation	c.305_306del	p.Q102Rfs*22			[5]
		c.1076-1080del	p.K359Rfs*14			[1]
		c.1084_1085del	p.R362Dfs*12			[2]
		c.1386_1390del	p.D462Efs*2			
		c.2130dupC	p.N711Qfs*33			
		c.2187_2198delinsGT	p.G730Cfs*60			
		c.2250del	p.G751Dfs*42			
		c.2565_2568del	p.N857Afs*17			
		c.2647_2648dup	p.V884Sfs*12			
		c.2128_2131dup	N711Tfs*34			[6]
	Missense mutations generating a splice variant paired with a Zα domain P193A or P193L mutation	c.2763-2A>G	Splice variant	c.577C>G	p.P193A	[2]
		c.3020-3C>G				
		c.2271-3A>G		c.578C>T	p.P193L	[4]
IV	**Miscellaneous**					
	Homozygous missense mutation in dsRNA binding motif 1	c.1622T>A	p.I541A	c.1622T>A	p.I541A	[7]
	Missense mutation in the ADAR1-p150 start codon and A-to-I editase domain	c.1A>G	p.M1?	c.3124C>T	p.R1042C	[5]
	Missense mutation generating a splice variant and nonsense mutation introducing a premature stop codon	c.3444-IG>A	splice variant	c.1600C>T	p.A534X	
	Frameshift mutation introducing a premature stop codon and missense mutation in the A-to-I editase domain	c.1493_1494delAG	p.Glu498ValfsX18	c.3577G>A	p.E1193K	[8]

Legend Supplementary Table 1. Type I interferonopathies such as Aicardi-Goutières Syndrome (AGS) comprise a subset of inflammatory diseases characterized by an increased interferon-stimulated gene (ISG) signature. Genetic mutations in *ADAR* is one of the nine genetic causes of AGS that have been identified so far. These mutations affect ADAR1 activity and can be divided into four categories. First, homozygous missense mutations in the A-to-I editase domain of ADAR1 (Category I) that (are predicted to) have a negative impact on the enzymatic activity of ADAR1. Second, glycine 1007 mutation to arginine (G1007R), which constitutes a heterozygous dominant-negative mutation (Category II) as it may inhibit the base-flipping process that delivers the substrate adenosine into the catalytic domain of ADAR1. A third group consists of compound heterozygous mutations combining a Zα domain-mutant allele with a second dysfunctional ADAR allele (Category III). Proline 193 is part of the ADAR1-p150-specific Zα domain and substitution of this residue with an alanine is the most common *ADAR* mutation found in patients with AGS. The second mutant allele either affects the A-to-I editase domain or abrogates ADAR1 expression owing to the introduction of a premature stop codon, frameshift or generation of a splice variant. Several other genetic compositions (Category IV) include homozygous missense mutations in the first double-stranded RNA-binding motif (dsRBM1) and the combination of an A-to-I editing-mutant allele with an *ADAR* or *ADAR-p150* null allele.

Supplementary Table 2 | Overview of *Adar* loss of function mouse models and genetic rescue

Model	Genetic background	Time of death	Ref.
ADAR1 deficient models			
Adar$^{-/-}$	Wild-type	*In utero* (E12.5)	[9,10,11]
		In utero (N.D.)	[12]
	Eif2ak2$^{-/-}$	*In utero* (N.D.)	[9,13]
	Zbp1$^{-/-}$	*In utero* (N.D.)	[12]
	Zbp1^{Zα1Zα2}	*In utero* (E13.5)	[11]
	Stat1$^{-/-}$	*In utero* (E15.5)	[14]
	Ifnar1$^{-/-}$/*Ifngr1*$^{-/-}$	*In utero* (E16.5)	[15]
	Ifnar1$^{-/-}$		[14]
	Mavs$^{-/-}$	Postnatal (P1)	
		Fully penetrant at P10	[11,12]
	Ifih1$^{-/-}$/*Eif2ak2*$^{-/-}$	~40% survivors post weaning	[13]
	Mavs$^{-/-}$/*Zbp1*^{Zα1Zα2}	Median survival of 20 weeks	[11]
	Mavs$^{-/-}$/*Zbp1*$^{-/-}$	~40% survivors post weaning	[12]
	Mavs$^{-/-}$/*Ripk3*$^{-/-}$		
Truncated ADAR1 models			
Adar$^{ΔEx7-9}$	Wild type	*In utero* (E12.5)	[10]
	Tmem173$^{-/-}$	*In utero* (N.D)	[16]
	Ddx58$^{-/-}$	*In utero* (N.D)	
	Irf3$^{-/-}$	*In utero* (N.D)	[17]
	Ifih1$^{-/-}$	Fully penetrant at P10	[16]
		Median survival of 20 days	[17]
	Mavs$^{-/-}$	Fully penetrant at P10	[16]
		Median survival of 15 days	[17]
Adar1-p110 deficient models			
Adar$^{p110-/-}$	Wild type	Median survival of 3 days	[18]
	Ifih1$^{-/-}$	Median survival of 2 days	
Adar$^{p110-/E861A}$	Wild-type	Normal viability	
Adar1-p150 deficient models			
Adar$^{p150-/-}$ (exon 1A)	Wild type	*In utero* (E12.5)	[19]
Adar^{L196CfsX6}			[20]
Adar$^{p150-/-}$ (exon 1A)	*Zbp1*$^{-/-}$	*In utero* (N.D)	[21]
	Ifih1$^{-/-}$	Fully penetrant at P5	
Adar^{L196CfsX6}		Median survival of 20 days	[13]
Adar$^{p150-/-}$ (exon 1A)	*Mavs*$^{-/-}$	Median survival > 20 days	[16]
	Ifih1$^{-/-}$/*Zbp1*$^{-/-}$	Median survival of 15 days	[21]
Adar^{L196CfsX6}	*Ifih1*$^{-/-}$/*Eif2ak2*$^{-/-}$	Normal viability	[13]
Deaminase deficient models			
Adar^{E861A}	Wild type	In utero (E14.5)	[22]
	Bax$^{-/-}$/*Bak*$^{-/-}$	*In utero* (N.D)	[23]
	Irf7$^{-/-}$		[24]
	Ifih1$^{-/-}$	Normal viability	[22]
Deaminase domain mutant models			
Adar^{K948N}	Wild type	Normal viability (N.D)	[25,26]
	Ifih1$^{-/-}$		[26]
Adar^{D963H}	Wild type		[27]
	Ifih1$^{-/-}$		
Zα domain mutant models			

Adar$^{N175A+Y179A}$	Wild type	Normal viability	[28,29]
	Mavs$^{-/-}$		
Adar$^{N175D+Y179A}$	Wild type		[12]
Adar$^{N175A+W197A}$		Median survival of 40 days	[30]
Adar^{P195A}		Normal viability	[31,32,33]
	Ifih1$^{-/-}$	Normal viability	[33]
Adar^{W197A}	Wild type	Median survival of 40 days	[30]
	Ifih1$^{-/-}$	Normal viability (N.D)	
Compound heterozygous mutation models			
Adar$^{N175A+Y179A/-}$	Wild type	Fully penetrant at P1	[11,28]
	Mavs$^{-/-}$		[28]
	Mavs$^{+/-}$/ *Zbp1*$^{Za1Za2/+}$	Median survival of 16 weeks	[11]
	Mavs$^{+/-}$/ *Zbp1*^{Za1Za2}	Normal viability	
	Zbp1$^{Za1Za2/+}$	Fully penetrant at P7	
	Zbp1^{Za1Za2}	Median survival of 20 weeks	
	Ripk3$^{-/-}$	Fully penetrant at P7	
	Mlkl$^{-/-}$	Fully penetrant at P1	
	Casp8$^{-/-}$/*Mlkl*$^{-/-}$		
Adar$^{N175D+Y179A/-}$	Wild type	Fully penetrant at P10	[12]
	Mavs$^{+/-}$	Median survival > 100 days	
	Mavs$^{-/-}$	Normal viability	
	Mavs$^{+/-}$ / *Zbp1*$^{-/-}$	Normal viability	
	Mavs$^{-/-}$ / *Zbp1*$^{-/-}$	Normal viability	
	Zbp1^{Za1Za2}	Median survival of 120 days	
	Zbp1$^{-/-}$	Median survival > 120 days	
	Ripk3$^{-/-}$	Fully penetrant at P10	
	Mlkl$^{-/-}$		
	Fadd$^{-/-}$/*Ripk3*$^{-/-}$		
	Fadd$^{-/-}$/*Mlkl*$^{-/-}$		
	Ripk1^{Rhim}/*Mlkl*$^{-/-}$		
Adar$^{P195A/\Delta Ex7-9}$	Wild type	Median survival of 4 weeks	[31]
	Ifih1$^{-/-}$	Normal viability	

Adar$^{P195A/p150-}$	Wild type	Median survival of 6 weeks	
	RnaseL$^{-/-}$	Normal viability	
	Ifih1$^{-/-}$	Normal viability	
	Dhx58$^{-/-}$	Normal viability	
	Ifnar1$^{-/-}$	Normal viability	
	Eif2ak2$^{-/-}$	Normal viability	
Adar$^{P195A/p150-}$	*Zbp1*$^{+/-}$	Median survival of 4 weeks	[21]
	Zbp1$^{-/-}$	Normal viability	
	Zbp1^{A64P}	Normal viability	
	Ripk3$^{+/-}$	Median survival of 4 weeks	
	Ripk3$^{-/-}$	Median survival of 18 weeks	
	Mlkl$^{+/-}$	Median survival of 4 weeks	
	Mlkl$^{-/-}$	Median survival of 4 weeks	
	Ripk1^{KD}	Median survival of 4 weeks	
	Casp8$^{+/-}$*/Ripk3*$^{-/-}$	Median survival of 7 weeks	
	Casp8$^{-/-}$*/Ripk3*$^{-/-}$	Median survival of 4 weeks	
Adar$^{P195A/-}$	Wild type	Median survival of 4 weeks	[31]
		Median survival of 17 weeks with 50% long term survivors	[32]
		Normal viability	[33]
	Ifih1$^{-/-}$	Normal viability	[32,33]
Adar$^{P195A/E861A}$	Wild type	Normal viability	[32]
Adar$^{W197A/E861A}$		Median survival of 3 weeks	[30]
Adar$^{K948N/E861A}$		P1	[26]
Adar$^{p110-/K948N}$		Normal viability	

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Legend Supplementary Table 2. *Adar*, adenosine deaminase acting on dsRNA (encoding ADAR1); *Eif2ak2*, eukaryotic translation initiation factor 2 alpha kinase 2 (encoding PKR); *Zbp1*, Z-DNA binding protein 1; $Zbp1^{Z\alpha1Z\alpha2}$, Z-DNA binding protein 1 with mutant Zα domains (N46A/D + Y50A and N122A/D + Y126A); *Stat1*, signal transducer and activator of transcription 1; *Ifnar1*, interferon alpha and beta receptor subunit 1; *Ifngr1*, interferon gamma receptor 1; *Ifih1*, interferon induced with helicase C domain 1 (encoding MDA5); *Mavs*, mitochondrial antiviral signaling protein; *Ripk3*, receptor interacting serine/threonine protein kinase 3; *Sting1*, stimulator of interferon response cGAMP interactor 1; *Ddx58*, DExD/H-Box helicase 58 (encoding RIG-I); *Irf3*, interferon regulatory factor 3; *Bax*, BCL-2-associated X, apoptosis regulator; *Bak*, BCL-2 antagonist/killer 1; *Irf7*, interferon regulatory factor 7; *Mlkl*, mixed lineage kinase domain like pseudokinase; *Casp8*, caspase-8; *Fadd*, Fas associated via death domain; $Ripk1^{RHIM}$, receptor interacting serine/threonine kinase 1 with mutated RIP homotypic interaction motif; *RnaseL*, ribonuclease L; *Dhx58*, DExH-Box helicase 58 (encoding LGP2); $Ripk1^{KD}$, receptor interacting serine/threonine protein kinase 1 with mutated kinase domain (K45A).

Supplementary Table 3 | Phenotypic overview of the *Adar* conditional knockout mouse models

Adar model	Cre model	Target	Reported phenotype	(Partial) rescue	Ref
ΔEx12-15	Albumin-Cre	Hepatocytes	Early postnatal lethality due to severe liver inflammation, impaired hepatocyte differentiation, apoptosis and fibrosis	N.D.	[9,34]
ΔEx7-9					[35]
ΔEx12-15	*Cdh5*-Cre	Endothelial cells	Early postnatal lethality due to multi-organ inflammation, including defects in lung, liver, intestine and kidney homeostasis	*Ifih1*$^{-/-}$	[36]
ΔEx12-15	*Xmlc2*-Cre	Developing cardiomyocytes	No overt phenotype	N.D.	[24]
ΔEx12-15	*Nkx2.5*-Cre		Embryonic lethal with severe cardiac inflammation and cell death (E18.5)		[37]
ΔEx12-15	α-*Mhc*-Cre	Cardiomyocytes	Myocarditis with ISG signature and lethal heart failure starting at 6 months of age	*Ifih1*$^{-/-}$	[24]
				Irf7$^{-/-}$	
ΔEx12-15 + E861A			No overt phenotype	*Ifih1*$^{-/-}$	
ΔEx12-15			Acute ADAR1 depletion results in rapid lethality due to severe cardiac inflammation	Salubrinal	[38]
ΔEx12-15	*Ptf1a*-Cre	Pancreas	Early postnatal lethality due to pancreatic atrophy, inflammation and cell death	*Mavs*$^{-/-}$	[39]
ΔEx7-9	Mip-Cre-ER	Pancreatic β cells	Acute ADAR1 depletion induced diabetes due to islet inflammation and β cell dysfunction	N.D.	[40]
ΔEx12-15	*Mist1*-Cre	Gastric chief cells	Loss of ADAR1 abrogates gastric metaplasia by impaired chief cell proliferation and cell death	N.D.	[41]
ΔEx12-15	HtPA-Cre	Neural crest cells	Early postnatal lethality with rapid loss of melanin producing melanocytes and myelin producing Schwann cells	*Ifih1*$^{-/-}$	[42]
	Wnt1-CRe		Complete penetrance of lethality on the day of birth	N.D.	
ΔEx7-9	Cyp19a1-Cre	Granulosa cells	Altered oocyte development, ovulation failure leading to infertility	N.D.	[43]
ΔEx12-15	K14-Cre-ER	Epidermal stem cells	Acute ADAR1 depletion results in severe skin inflammation with loss of hair follicles and melanocytes	N.D.	[44]
ΔEx7-9 + ΔEx2-13	Epor-Cre	Erythrocytes	Embryonic lethal due to the progressive loss of red blood cells	N.D.	[45]
ΔEx7-9 + ΔEx2-13	LysM-Cre	Myeloid cells	Loss of ADAR1 does not have an overt effect on myelopoiesis	N.D.	[45]
ΔEx7-9			Loss of ADAR1 yields dysfunctional alveolar macrophages		[46]
			Alleviation of colon tumorigenesis due to spontaneous ZBP1 activation in myeloid cells		[47]
ΔEx7-9	CD11c-Cre	CD11c+ APCs	Enrichment of inflammatory cDC2-like cells and an increased number of activated tissue resident memory T cells in the lung	*Ifnar1*$^{-/-}$	[48]
			Systemic loss of CD103+ dendritic cells and loss of alveolar macrophages	N.D.	[46]
ΔEx12-15	CD4-Cre	CD4+/CD8+ double positive T cells	Impaired T cell maturation and failed negative selection induces spontaneous colitis	*Ifih1*$^{-/-}$	[49]
ΔEx12-15	Lck-Cre	CD4-/CD8- double negative T cells	Absence of ADAR1 induces cell death of DN T cells and abrogates TCR expression	N.D.	[50]
			Combined MDA5 deficiency and forced TCR expression restores T cell maturation	*Ifih1*$^{-/-}$*/HY-TCR*	[51]
ΔEx7-9	*Mb1*-Cre	< Fr.B stage B cells	Absence of ADAR1 induces cell death of early stage B cells and abrogates BCR expression	*Ifih1*$^{-/-}$*/MD4*	[52]
	CD19-Cre	> Fr.E stage B cells	Breached B cell maturation induces depletion of immature and CD23+ mature recirculating B cells	N.D.	[53]
ΔEx12-15	*Aicda*-Cre	Activated B cells	Loss of germinal center B cells and a defective T cell dependent Ab response	N.D.	[54]

ΔEx12-15	Cre-ER TM	Inducible (full body)	Acute ADAR1 depletion results in decreased bone mass due to impaired osteoblast differentiation without affecting osteoclasts	N.D.	[55]
ΔEx7-9	CAGG-Cre-ER		Acute ADAR1 depletion induces severe intestinal inflammation due to loss of cycling intestinal stem cells	Salubrinal	[56]
	UBC-Cre-ERT2		Acute ADAR1 depletion induces intestinal shortening despite MAVS deficiency	*Mavs*$^{-/-}$	[16]
	Rosa26-Cre-ERT2		Acute ADAR1 depletion does not induce intestinal shortening in the absence of MDA5	*Ifih1*$^{-/-}$	[57]
ΔEx7-9 + ΔEx2-13	SCL-Cre-ERT		In the absence of ADAR1 cycling hematopoietic stem cells undergo apoptosis	N.D.	[58]
ΔEx12-15	MSCV-Cre		ADAR1 deficient HSCs are capable of homing, but fail to reconstitute blood lineages upon transplantation	N.D.	[59]
ΔEx7-9	Rosa26-Cre-ERT2		Rapid lethality due to hematopoietic defects	*Ifih1-/-*	[57]
ΔEx7-9 + E861A					
ΔEx7-9 + L196CfsX6				N.D.	
ΔEx7-9 + P195A			No lethality or overt hematopoietic defects		[32]

Legend Supplementary Table 3. N.D., not determined; *Adar*, adenosine deaminase acting on dsRNA (encoding ADAR1); *Cdh5*, cadherin 5; XMLC2, *Xenopus laevis* myosin light-chain 2; *Nkx2.5*, NK2 homeobox 5; E, embryonic day; α*MyHC*, α-myosin heavy chain; ISG, interferon-stimulated gene; *Ifih1*, interferon induced with helicase C domain 1 (encoding MDA5); *Irf7*, interferon regulatory factor 7; *Ptf1a*, pancreas associated transcription factor 1a; *Mavs*, mitochondrial antiviral signalling protein; Mip, mouse insulin I promoter; *Mist1*, muscle, intestine and stomach expression 1; *htPA*, human tissue plasminogen; *Wnt1*, Wnt family member 1; *Cyp19a1*, cytochrome P450 family 19 subfamily A member 1; K14, human keratin 14 promotor; $Ripk1^{FL/FL}$, receptor interacting serine/threonine protein kinase 1 floxed; K5, bovine keratin 5 promotor; *Epor*, erythropoietin receptor; *LysM*, lysozyme M; *Cd11c*, cluster of differentiation 11c; APC, antigen-presenting cell; cDC2, conventional dendritic cell type 2; *Ifnar1*, interferon alpha and beta receptor subunit 1; *Cd4*, cluster of differentiation 4; *Lck* , LCK proto-oncogene, Src family tyrosine kinase; DN, double-negative; TCR, T cell receptor; *Mb1*, immunoglobulin-alpha subunit of the B lymphocyte antigen receptor; BCR, B cell receptor; *Cd19*, cluster of differentiation 19; *Cd23*, cluster of differentiation 23; *Aicda*, activation induced cytidine deaminase; Ab, antibody; ER, estrogen receptor; TM, tamoxifen; UBC, ubiquitin C; SCL, stem cell leukemia; MSCV, murine stem cell virus; HSC, haematopoietic stem cell.

Supplementary Table References

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