ADAR1 Facilitates HIV-1 Replication in Primary CD4+ T Cells

Eloy Cuadrado1*, Thijs Booiman1,2*, John L. van Hamme1,2, Machiel H. Jansen1, Karel A. van Dort1,2, Adeline Vanderver3, Gillian I. Rice4, Yanick J. Crow4,5, Neeltje A. Kootstra1,2, Taco W. Kuijpers1,6

1 Department of Experimental Immunology, Academic Medical Center (AMC), University of Amsterdam (UvA), Amsterdam, The Netherlands, 2 Sanquin Research, Landsteiner Laboratory and Center for Infection and Immunity (CINIMA) at the Academic Medical Center of the University of Amsterdam, Amsterdam, The Netherlands, 3 Center for Genetic Medicine Research, Children’s National Medical Center, Washington DC, United States of America, 4 Manchester Centre for Genomic Medicine, University of Manchester, Manchester Academic Health Sciences Centre (MAHSC), Manchester, United Kingdom, 5 INSERM UMR 1163, Laboratory of Neurogenetics and Neuroinflammation, Institut Imagine, Hôpital Necker, Paris, France, 6 Emma Children’s Hospital, Dept of Pediatric Hematology, Immunology and Infectious disease, AMC, UvA, Amsterdam, The Netherlands

These authors contributed equally to this work.
* e.cuadrado@sanquin.nl

Abstract

Unlike resting CD4+ T cells, activated CD4+ T cells are highly susceptible to infection of human immunodeficiency virus 1 (HIV-1). HIV-1 infects T cells and macrophages without activating the nucleic acid sensors and the anti-viral type I interferon response. Adenosine deaminase acting on RNA 1 (ADAR1) is an RNA editing enzyme that displays antiviral activity against several RNA viruses. Mutations in ADAR1 cause the autoimmune disorder Aicardi-Goutiéres syndrome (AGS). This disease is characterized by an inappropriate activation of the interferon-stimulated gene response. Here we show that HIV-1 replication, in ADAR1-deficient CD4+ T cells from AGS patients, is blocked at the level of protein translation. Furthermore, viral protein synthesis block is accompanied by an activation of interferon-stimulated genes. RNA silencing of ADAR1 in Jurkat cells also inhibited HIV-1 protein synthesis. Our data support that HIV-1 requires ADAR1 for efficient replication in human CD4+ T cells.

Introduction

Recent studies have begun to elucidate the role of host proteins in the innate immune response to infection with HIV-1. Of particular note, HIV-1 exploits the three prime repair exonuclease 1 (TREX1) protein to avoid the induction of type I interferons in CD4+ T cells and macrophages [1], whilst the deoxynucleoside triphosphohydrolase (SAMHD1) has been identified as a restriction factor limiting HIV-1 infection of myeloid lineage cells [2–4]. The genes encoding TREX1 [5] and SAMHD1 [6] are mutated in the human type I interferonopathy Aicardi-Gouтиёres syndrome (AGS). AGS is a rare genetic autoimmune disorder that can mimic congenital
viral infection [5] and is characterized by an excessive production of interferon alpha (IFN-α) [7]. AGS can also be caused by dysfunction of the RNase-H2 endonuclease complex [8], the cytosolic double-stranded RNA (dsRNA) sensor MDA5 [9,10] and the dsRNA-editing enzyme ADAR1 [11]. All these proteins are important in nucleic acid metabolism and mutations in one of the AGS genes, i.e. TREX1, have been shown to lead to the accumulation of nucleic acids in cells, mimicking a viral infection and to the activation of the innate immune response [12–14].

ADAR1 activity has been studied in different types of viral infections, and both pro-viral [15,16] and anti-viral [17–19] activities have been reported and have been thoroughly reviewed elsewhere [20]. A highly selective editing of viral RNA mediated by ADAR1 has been demonstrated for hepatitis C virus (HCV) [19]. For HIV-1, the existing data are scarce and sometimes conflicting. ADAR1 has been reported to facilitate HIV-1 replication [21–23], whereas another study suggested that ADAR1 inhibits the production of viral proteins [24]. None of these investigations were carried out in human CD4+ T cells and thus little is known about the exact involvement of ADAR1 in the life cycle of HIV-1 in human CD4+ T cells.

**Methods**

**Patient recruitment and cell isolation**

AGS patients were recruited at Children’s National Medical Center, Washington DC, USA; Manchester Academic Health Science Centre, Manchester, UK; and the Academic Medical Center, Amsterdam, The Netherlands. ADAR1 mutation status and patient demographics have been described previously [11] and the information is summarized in Table 1. This study was conducted in accordance with the ethical principles set out in the declaration of Helsinki, written informed consent was obtained from each patient, and the Medical Ethics Committee of the Academic Medical Center and the Ethics Advisory Body of the Sanquin Blood Supply Foundation in Amsterdam (MEC07/234) approved the study.

**Cell culture**

PBMC were obtained from buffy coats from healthy blood donors. PBMC were isolated by Ficoll-Isopaque density gradient centrifugation and cultured in Iscove’s modified Dulbecco medium (IMDM) supplemented with 10% (v/v) heat-inactivated fetal calf serum (FCS), penicillin (100 U/ml), streptomycin (100 μg/ml) and IL-2 (100 IU/ml) and maintained in a humidified 10% CO2 incubator at 37°C.

We used Jurkat T cells expressing CCR5 generated by retroviral transduction as previously published [25,26]. Cells were cultured in Roswell Park Memorial Institute Medium (RPMI) (Lonza, Basel, Switzerland) supplemented with 10% (v/v) heat-inactivated fetal calf serum (FCS), penicillin (100 U/ml) and streptomycin (100 μg/ml), in a humidified 5% CO2 incubator at 37°C. When indicated, cells were treated with a cocktail of RTIs including 10 μM AZT, 20 μM DDI, and 10 μM 3TC.

**Table 1. Demographic data, ancestry, and sequence alterations in ADAR1 mutation–positive donors.**

| Patient | Age | Sex | Ancestry                  | Consanguinity | Nucleotide alteration | Exon | Amino-acid alteration |
|---------|-----|-----|----------------------------|---------------|-----------------------|------|----------------------|
| Patient 1 | 14  | F   | Norwegian                 | Non-consanguine | c.[577C>G]+[2675G>A] | 2, 9 | p.[Pro193Ala]+[Arg892His] |
| Patient 2 | 4   | M   | European American         | Non-consanguine | c.3019G>A (het, de novo) | 11   | p.Gly1007Arg         |
| Patient 3 | 9   | F   | Pakistani                 | Consanguine    | c.3337G>C (hom)       | 14   | p.Asp1113His         |

M, male; F, female; het, heterozygous; hom, homozygous.

doi:10.1371/journal.pone.0143613.t001
Viruses
HIV-1(YU2, HIV-1(YU2-GFP, and VSV-G pseudotyped NL4-3.Luc.R-E-luciferase reporter viruses were produced by transfection of respectively pYU2, pYU2-GFP, or pNL4-3.Luc.R-E- in combination with pCMV-VSV-G in HEK293T cells. Lentiviral vectors (LV) were produced by co-transfection of pCMV-VSV-G, pMDLgp and pRSV-Rev and pLKO.1 constructs expressing ADAR1 shRNA in HEK293T cells. The following plasmids targeting ADAR1 were obtained from the The RNAi Consortium (TRC) library (Sigma, St Louis, MO, USA): TRCN0000050788 (clone d10), TRCN0000050789 (clone d11), TRCN0000050790 (clone d12), TRCN0000050791 (clone e1), TRCN0000050792 (clone e2). Vectors containing a non-targeting (NT) sequence of the human genome (NT-shRNA, SHC002, MISSION® Non-Target shRNA Control Vector) or green fluorescent protein (SHC003, MISSION® TurboGFP Control Vector) from Sigma were used as controls. Transfections were performed with the calcium phosphate method as described previously [27]. In short, plasmid DNA was diluted in 0.042M HEPES containing 0.15M CaCl2, subsequently mixed with an equal volume of 2× HEPES buffered saline pH 7.2, incubated at room temperature for 15 min and added to the culture medium. After 24h incubation in a humidified 3% CO2 incubator at 37°C, the culture medium was replaced and cultures were continued at 10% CO2 at 37°C. Virus was harvested at 48 and 72h after transfection and passed through a 0.22 μm filter. HIV-1 virus titers were quantified by determining the TCID50 on TZMBL or HEK293T. Lentiviral vector supernatant was concentrated by centrifugation at 50,000xg for 2.5h directly after harvesting. Equal amounts of lentiviral vector as determined by p24 ELISA were used to inoculate target cells.

Lentiviral transduction and HIV-1 infection
To obtain Jurkat T cells with a stable ADAR1 knockdown, the cells were inoculated with either the LV-shRNA NT-control or the LV-shRNA targeting ADAR1 at a MOI of 10. Cells were subsequently cultured in the presence of puromycin (2 μg/ml) for several passages to obtain a stable transduced cell line. Jurkat T cell lines and IL-2 stimulated PBMCs were infected with YU2, YU2-GFP or the VSV-G pseudotyped NL4-3.Luc.R-E-luciferase reporter viruses which were treated with DNase (200 ng/ml) (Promega, Madison WI, USA) for 45 min at 37°C in medium containing 6 mM MgCl2 at a MOI of 0.1 and 1. Three hours post-infection the cells were washed with medium and the medium was replaced. pol proviral DNA copies and pol, Gag and GFP mRNA levels were analysed 48h post infection. Gag p24 levels in the culture supernatant were determined respectively 7 days post infection by an in-house enzyme-linked immunosorbent assay [28]. Luciferase activity was determined 48h post infection as a measure for HIV-1 replication, by adding 25 μl substrate (0.83 mM ATP, 0.83 mM D-luciferin (Duchefa, Haarlem, The Netherlands), 18.7 mM MgCl2, 0.78 μM Na2H2P2O7, 38.9 mM Tris pH 7.8, 0.39% (v/v) glycerol, 0.03% (v/v) Triton X-100, and 2.6 μM dithiothreitol) directly to the culture medium. Luminescence was measured for 1 s per well using a luminometer (Berthold, Bad Wildbad, Germany).

Nucleic acid isolation and quantitative PCR
Total DNA and RNA were isolated using the Allprep DNA/RNA mini kit (QIAGEN). Reverse transcription of mRNA was performed using the Roche Transcriptor First Strand cDNA Synthesis kit (Roche) using a maximum of 1 μg total RNA and random hexamer primers. The primers used for qPCR and real time qPCR were ADAR1 p110 fw 5’-GCGAGGCCCTCCGGG TG-3, rv 5’-CTGTCCTGTGCTCATAGCCTTGTA-3’; ADAR1 p150 fw 5’-CGGGCAATGCGC-3, rv 5’- AATGGATGGGTGTAGTATCCGC-3; GAPDH fw 5’TGCACACCAAC TGCTTAGC-3’, rv 5’- GCCATGGACTGTGTCATGA-3’; ACTB fw 5’-GCTCCTCCTGTA
ADAR1 Facilitates HIV-1 Replication in Primary CD4+ T Cells

Flow cytometry
Expression of surface-bound receptors and viral GFP was detected by flow cytometry, with the commercially available monoclonal antibodies 7-AAD, CD3 Pacific Blue, and CD4 PE-Cy7 or V500 (BD Biosciences, San Jose, CA). Samples were analyzed on a FACS Canto II flow cytometer equipped with FACSDiva software (BD Biosciences, San Jose, CA, USA). Cells were gated based on their forward and side scatter, and 20,000 gated events were collected per sample. Data were analyzed using FlowJo Version 7.6.4 software (TreeStar).

Western blot
Cells were lysed in RIPA buffer (150 mM NaCl, 1% Triton X-100, 0.5% sodium deoxycholate, 0.1% SDS, 50 mM Tris, pH 8.0) containing Complete EDTA free protease inhibitor (Roche). After adding NuPAGE LDS 4x sample buffer (Invitrogen) and 0.1 M DTT, samples were heated at 95°C for 10 min. The Odyssey Protein Weight Marker was used as a size reference (LI-COR, Lincoln, NE, USA). Proteins were separated by SDS-PAGE (NuPAGE 10% Bis-Tris precast gel and MES SDS running buffer (Invitrogen) and transferred to a nitrocellulose membrane (Protran, Schleicher & Schuell, Dassel, Germany) using NuPAGE transfer buffer. After blocking for 2h with PBS containing 5% Protifar (Nutricia, Schiphol, The Netherlands) and 0.5% bovine serum albumin, the blot was incubated with anti ADAR1 antibody to detect ADAR1 (1:1000; Sigma-Aldrich St. Louis, MO, USA), eIF2α (1:1000; mAb #2103, Cell Signalling, Beverly, MA, USA), Phospho-eIF2α (Ser51) (1:1000; mAb #3597, Cell Signalling, Beverly, MA, USA), and SC-1616 anti-β-actin antibody (1:200; Santa Cruz Biotechnology, Santa Cruz, CA, USA) to detect β-actin. IRDye 680 conjugated Donkey anti-Rabbit IgG (1:15000; 926–32223, LI-COR, Lincoln, NE, USA) and IRDye 680LT conjugated Donkey anti-Goat IgG (1:15000; 926–32224, LI-COR) were used as secondary antibodies to visualize expression using the Odyssey infrared image system (LI-COR). Image J software was used to quantify protein expression.

Statistics
Data were analyzed using GraphPad Prism 6.0 (GraphPad Software Inc., San Diego, CA, USA). All experiments were performed at least 3 independent times, unless otherwise specified. The variable distribution was assessed by the Kolmogorov-Smirnov test. When test distribution was normal a Student t-test was used to determine differences between 2 groups. When distribution was not normal, a non-parametric Mann-Whitney test was used to determine
Results and Discussion

Here we have studied the role of ADAR1 in HIV-1 replication using primary T cells obtained from AGS patients carrying known ADAR1 mutations [11], and thus lacking fully functional ADAR1 activity. Infection of CD3+CD4+ T cells with the HIV-1 GFP reporter virus was analyzed by flow cytometry (Fig 1A–1C). CD4+ T cells from healthy donors supported productive infection with HIV-1 GFP, yielding >2.5% of GFP positive CD3+CD4+ T cells 5 days post-infection (Fig 1D). In contrast, CD4+ T cells from AGS patients were refractory to infection with HIV-1 GFP. As expected, viral infection of CD4+ T cell cultures was inhibited in the presence of reverse transcriptase inhibitors (RTI) (Fig 1C). Infection of peripheral blood mononuclear cells (PBMCs) from AGS patients with a VSV-G pseudotyped luciferase reporter virus was also absent when compared to the healthy controls (Fig 1E), indicating that HIV-1 replication in PBMC from AGS patients is not restricted at the level of viral entry.

We determined which step of the viral replication cycle was impaired in cells from AGS patients. In agreement with the results described above, efficient viral replication in PBMC from healthy donors could be observed when virus production in the culture supernatant was analyzed using a p24 ELISA. However, p24 production in the culture supernatant from AGS PBMCs remained below the detection limit (Fig 1F). Next, we analyzed whether the process of HIV-1 reverse transcription and proviral transcription could occur efficiently in PBMC from ADAR1-deficient patients. We observed no differences in pol proviral DNA (Fig 1G) and pol mRNA levels (Fig 1H) in PBMC from AGS patients as compared to healthy controls. These data indicate that HIV-1 replication is impaired at a post-transcriptional step.

AGS is characterized by high levels of IFN-alpha, which usually invokes the activation of interferon-stimulated genes (ISGs) [29]. ISGs are found upregulated in PBMCs from patients carrying mutations in ADAR1 [11]. We also observed an upregulation in mRNA expression of ISG15, IFIT27, RSAD2, and OAS1 in AGS patient cells infected with HIV-1 compared to cells from healthy donors under similar conditions (Fig 1I).

To confirm that ADAR1 supports HIV-1 replication in T lymphocytes, we analyzed the effect of ADAR1 knockdown on HIV-1 replication in CCR5-expressing Jurkat T cells. Normally Jurkat cells do not express CCR5; for that reason we used a stable CCR5-expressing Jurkat cell line [30]. First, HEK293T cells were transduced with lentiviral vectors expressing a non-targeting (NT) control shRNA or shRNAs targeting ADAR1 gene transcription (Fig 2A and S1 Fig). Next, the most efficient ADAR1 shRNA clone (clone d11) was subsequently used on Jurkat T cells and resulted in a stable reduction of ADAR1 expression of 81% (Fig 2B and S1 Fig). These cells were infected with a HIV-1 GFP reporter virus at two different multiplicities of infection (MOIs), and viral replication was analyzed 5 days post-infection by flow cytometry (Fig 2C). We observed a decrease in the number of GFP+ cells and fluorescence intensity in the ADAR1 knockdown cells compared to NT control cells (Fig 2D and 2E). When these experiments were repeated with a replication-competent HIV-1 variant, we also observed a decrease in viral replication as measured by p24 release into the culture supernatant of these ADAR1 knockdown cells (Fig 2F). Similar results were obtained using a VSV-G pseudotyped virus (Fig 2G and 2H).

We then investigated which step of the viral replication cycle is facilitated by ADAR1. Proviral DNA and viral mRNA expression was determined by qPCR 48h post infection (MOI 0.1 and 1). We observed no difference in pol proviral DNA levels and pol mRNA expression in the ADAR1 knockdown cells when compared to the NT control cells (Fig 3A and 3B). In addition,
no differences were observed in Gag and GFP mRNA expression levels (Fig 3C and 3D). These results indicate that HIV-1 replication occurs efficiently in ADAR1 knockdown cells up to the point of viral transcription. When the level of Gag mRNA expression was correlated to the level of Gag protein expression, a clear decrease in protein production per viral gag transcript was observed in ADAR1 knockdown cells (Fig 3E) and this difference was already evident after 3 days post-infection (Fig 3F). Next, we analyzed ISG mRNA expression in CCR5-expressing Jurkat cells upon ADAR1 knockdown and observed no changes in ISG15, IFIT27, RSAD2, and...
OAS1 expression levels (Fig 3G). These data, together with the data from the primary cells obtained from the AGS patients, suggest that these ISGs are not responsible for the block in HIV-1 replication observed in ADAR1 deficient cells.

The actual mechanism by which ADAR1 interferes with HIV-1 replication is not fully understood. ADAR1 prevents phosphorylation of RNA-activated protein kinase (PKR) and thus phosphorylation of eukaryotic translation initiation factor 2α (eIF2α), which is important for the initiation of mRNA translation [23]. Here we show that mutations in ADAR1 or ADAR1-knockdown cells restrict HIV-1 replication at the level of protein translation, possibly through the inhibition of PKR and eIF2α phosphorylation. However, in our CCR5-expressing Jurkat T cells, the inhibition of HIV-1 replication could not be related to increasing levels of phosphorylated eIF2α upon ADAR1 knockdown as measured by western blot 2 days after infection (Fig 3H and S2 Fig). Nonetheless, the lack of increased phosphorylation of eIF2α at such early time point does not exclude PKR inhibition as the main key player in ADAR1-mediated blocking of HIV-1 virus replication. In fact, it has recently been shown that the PKR...

Fig 2. Silencing ADAR1 restricts HIV-1 replication in Jurkat T cells. (a) Jurkat T cells were stable transduced with shRNA control, GFP control or ADAR1 shRNAs. Bar graph shows normalized ADAR1 mRNA levels. Panel shows western blot of the two best shRNA targeting ADAR1. (b) Cells were transduced at MOI 10 or 20. Panels show representative western blot of ADAR1 protein reduction in ADAR1 shRNA transduced cells. Bar graphs show densitometry analysis of bands compared to Control shRNA. Actin was used as loading control. (c) Control shRNA (n = 4) and ADAR1 shRNA (n = 4) transduced Jurkat T cells were challenged with equivalent infectious units of HIV-1 GFP virus (MOI 0.1 or 1) and analyzed by FACS on day 5 after infection. Panels show representative dot plots of FACS analysis from transduced cells. The viable cells (gate, left graphs) were analyzed for GFP fluorescence. Histograms show GFP signal of Control shRNA (black line) and ADAR1 shRNA cells (red line) after HIV1-GFP infection at different MOIs. (d) Bar graph representing the mean ± s.d. of GFP+ infected cells (n = 4), **P<0.01. (e) Bar graph representing the mean ± s.d. of GFP fluorescence of infected cells (n = 4), **P<0.01. (f) Bar graphs represent mean ± s.d. of HIV-1 p24 protein amounts released into the cell culture supernatant on day 5 after infection, *P<0.05. (g) Histograms show GFP signal of control shRNA (black line) and ADAR1 shRNA cells (red line) after VSV-G-GFP infection at different MOIs. (h) Bar graph representing the mean ± s.d. of GFP+ infected cells (n = 3) **P<0.01, *P<0.05.

doi:10.1371/journal.pone.0143613.g002
activator (PACT) contributes to PKR inhibition during HIV-1 replication and that the TAR RNA binding protein (TRBP) interacts with ADAR1 resulting in an increased replication of HIV [31].

Our results undoubtedly show a clear decrease in Gag protein with no decrease in Gag mRNA. This may indicate a post-transcriptional regulation of Gag, which can occur through RNA editing or decreased translation. This latter phenomenon is compatible with the inhibition of PKR activation that has been described for HIV [23,31] but also for other viruses such as VSV [32], Measles virus [33,34] and human T-cell lymphotropic virus (HTLV-I) [35].

Fig 3. Relative quantification (mean±s.e.m) of viral DNA pol (a) and viral pol mRNA (b) expressed as fold versus NT-shRNA control cells (n = 3). (c) Gag (n = 2) and (d) GFP (n = 3) mRNA expressed as fold versus NT-shRNA control cells (e) Bar graphs show the ratio of Gag protein versus Gag mRNA levels on infected cells (n = 3), *p<0.05. (f) Time course ratio expression of Gag protein versus Gap mRNA from day 1 to day 5 after infection (n = 2 =). (g) Relative quantification of ISG15, IFIT27, RSAD2, IFIT44L, and OAS1 of control shRNA and ADAR1 shRNA cells. (h) Western blot of eIF2α and the phosphorylated form of eIF2α. Actin was used as loading control.

doi:10.1371/journal.pone.0143613.g003
ADAR1 activity through RNA editing has also been described for HIV [21,22] but also for other viruses such as equine infectious anemia virus (EIAV) [15].

In summary, our results demonstrate that ADAR1 facilitates HIV-1 replication in human primary CD4+ T cells by supporting efficient viral protein synthesis, clarifying the discrepancy of previous studies [20–24,31]. Unlike TREX1 exonuclease, which attenuates the innate immune response to HIV-1 by clearing viral DNA transcripts [1], ADAR1 seems to facilitate HIV-1 replication by permitting efficient viral protein synthesis. Our study demonstrates a role for ADAR1 in HIV-1 replication in primary CD4+ T cells and may contribute to the development of therapeutics against HIV/AIDS by targeting a novel cellular pathway important for HIV-1 replication.

Supporting Information

S1 Fig. Original ADAR1 Western Blots.
(TIFF)

S2 Fig. Original eIF2alpha Western Blots.
(TIFF)

Acknowledgments

We are grateful to Theo Geijtenbeek and Ramin Sarrami (Department of Experimental Immunology, Academic Medical Center Amsterdam) for kindly providing the CCR5-expressing Jurkat T cells.

Author Contributions

Conceived and designed the experiments: EC TB NAK TWK. Performed the experiments: EC TB JLH MHJ KAD. Analyzed the data: EC TB JLH MHJ NAK TWK. Contributed reagents/materials/analysis tools: AV GIR YJC. Wrote the paper: EC TB NAK TWK AV GIR YJC.

References

1. Yan N, Regalado-Magdos AD, Stiggelbout B, Lee-Kirsch MA, Lieberman J. The cytosolic exonuclease TREX1 inhibits the innate immune response to human immunodeficiency virus type 1. Nat Immunol. 2010; 11: 1005–1013. doi:10.1038/ni.1941 PMID: 20871604

2. Baldauf H-M, Pan X, Erikson E, Schmidt S, Daddacha W, Burggraf M, et al. SAMHD1 restricts HIV-1 infection in resting CD4+ T cells. Nature Medicine. 2012; 18: 1682–1689. doi:10.1038/nm.2964 PMID: 22972397

3. Berger A, Sommer AFR, Zwarg J, Hamdorf M, Welzel K, Esly N, et al. SAMHD1-Deficient CD14+ Cells from Individuals with Aicardi-Goutières Syndrome Are Highly Susceptible to HIV-1 Infection. PLoS Pathog. 2011; 7.

4. Ryoo J, Choi J, Oh C, Kim S, Seo M, Kim S-Y, et al. The ribonuclease activity of SAMHD1 is required for HIV-1 restriction. Nat Med. 2014

5. Crow YJ, Hayward BE, Parmar R, Robins P, Leitch A, Ali M, et al. Mutations in the gene encoding the 3'–5' DNA exonuclease TREX1 cause Aicardi-Goutières syndrome at the AGS1 locus. Nat Genet. 2006; 38: 917–920. doi: 10.1038/ng1845 PMID: 16845398

6. Rice GI, Bond J, Asipu A, Brunette RL, Manfield IW, Carr IM, et al. Mutations involved in Aicardi-Goutières syndrome implicate SAMHD1 as regulator of the innate immune response. Nat Genet. 2009; 41: 829–832. doi: 10.1038/ng.373 PMID: 19525950

7. Lebon P, Badoual J, Ponsot G, Goutières F, Hémeury-Cukier F, Aicardi J. Intrathecal synthesis of interferon-alpha in infants with progressive familial encephalopathy. J Neurol Sci. 1988; 84: 201–208. PMID: 2837539

8. Crow YJ, Leitch A, Hayward BE, Garner A, Parmar R, Griffith E, et al. Mutations in genes encoding ribonuclease H2 subunits cause Aicardi-Goutières syndrome and mimic congenital viral brain infection. Nature Genetics. 2006; 38: 910–916. doi: 10.1038/ng1842 PMID: 16845400
9. Oda H, Nakagawa K, Abe J, Awaysa T, Funakibi M, Hijiakata A, et al. Aicardi-Goutières Syndrome Is Caused by IFIH1 Mutations. Am J Hum Genet. 2014; 95: 121–125. doi: 10.1016/j.ajhg.2014.06.007 PMID: 24995871

10. Rice GI, Del Toro Duany Y, Jenkinson EM, Forte GMA, Anderson BH, Ariaudo G, et al. Gain-of-function mutations in IFIH1 cause a spectrum of human disease phenotypes associated with upregulated type I interferon signaling. Nat Genet. 2014; 46: 503–509. doi: 10.1038/ng.2933 PMID: 24686847

11. Rice GI, Kasher PR, Forte GMA, Manning NM, Greenwood SM, Szynkiewicz M, et al. Mutations in ADAR1 cause Aicardi-Goutières syndrome associated with a type I interferon signature. Nat Genet. 2012; 44: 1243–1248. doi: 10.1038/ng.2414 PMID: 23001123

12. Yang Y-G, Lindahl T, Barnes DE. Trex1 exonuclease degrades ssDNA to prevent chronic checkpoint activation and autoimmune disease. Cell. 2007; 131: 873–886. doi: 10.1016/j.cell.2007.10.017 PMID: 18045533

13. Stetson DB, Ko JS, Heidmann T, Medzhitov R. Trex1 prevents cell-intrinsic initiation of autoimmunity. Cell. 2008; 134: 587–598. doi: 10.1016/j.cell.2008.06.032 PMID: 18724932

14. Gall A, Treuting P, Elkon KB, Loo Y-M, Barber GN, et al. Autoimmunity Initiates in Nonhematopoietic Cells and Progresses via Lymphocytes in an Interferon-Dependent Autoimmune Disease. Immunity. 2012; 36: 120–131. doi: 10.1016/j.immuni.2011.11.018 PMID: 22284419

15. Tang Y-D, Na L, Fu L-H, Yang F, Zhu C-H, Tang L, et al. Double-stranded RNA-specific adenosine deaminase 1 (ADAR1) promotes EIAV replication and infectivity. Virolology. 2015; 476: 1926–1936. doi: 10.1016/j.virol.2014.12.038 PMID: 25589239

16. Gélinas J-F, Clerzius G, Shaw E, Gatignol A. Enhancement of replication of RNA viruses by ADAR1 via RNA editing and inhibition of RNA-activated protein kinase. J Virol. 2011; 85: 4860–4866. doi: 10.1128/JVI.00240-11 PMID: 21490091

17. Polson AG, Ley HL 3rd, Bass BL, Casey JL. Hepatitis delta virus RNA editing is highly specific for the amber/W site and is suppressed by hepatitis delta antigen. Mol Cell Biol. 1998; 18: 1919–1926. PMID: 9528763

18. Sato S, Wong SK, Lazinski DW. Hepatitis delta virus minimal substrates competent for editing by ADAR1 and ADAR2. J Virol. 2001; 75: 8547–8555. PMID: 11507200

19. Taylor DR, Puig M, Darnell MR, Mihalik K, Feinstone SM. New antiviral pathway that mediates hepatitis C virus replicon interferon sensitivity through ADAR1. J Virol. 2005; 79: 6291–6298. doi: 10.1128/JVI.79.10.6291–6298.2005 PMID: 15858013

20. Samuel CE. Adenosine deaminases acting on RNA (ADARs) are both antiviral and proviral. Virology. 2011; 411: 180–193. doi: 10.1016/j.virol.2010.12.004 PMID: 21218111

21. Phuphuakrat A, Kraitong K, Loo Y-M, Barber GN, et al. Double-stranded RNA editing and inhibition of RNA-activated protein kinase. J Virol. 2008; 82: 10864–10872. doi: 10.1128/JVI.00238-08 PMID: 18753201

22. Doria M, Neri F, Gallo A, Farace MG, Michienzi A. Editing of HIV-1 RNA by the double-stranded RNA deaminase ADAR1 stimulates viral infection. Nucleic Acids Res. 2009; 37: 5848–5858. doi: 10.1093/nar/gkp604 PMID: 19651874

23. Clerzius G, Gélinas J-F, Daher A, Bonnet M, Meurs EF, Gatignol A. ADAR1 interacts with PKR during human immunodeficiency virus infection of lymphocytes and contributes to viral replication. J Virol. 2009; 83: 10847–10855. doi: 10.1128/JVI.00240-11 PMID: 19605474

24. Biswas N, Wang T, Ding M, Tumme A, Chen Y, Wang Q, et al. ADAR1 is a novel multi targeted antivi-H1-1 cellular protein. Virolology. 2012; 422: 265–277. doi: 10.1016/j.virol.2011.10.024 PMID: 22104209

25. Arrighi J-F, Pion M, Wzinerowicz M, Geijtenbeek TB, Garcia E, Abraham S, et al. Lentivirus-mediated RNA interference of DC-SIGN expression inhibits human immunodeficiency virus transmission from dendritic cells to T cells. J Virol. 2004; 78: 10848–10855. doi: 10.1128/JVI.78.20.10848–10855.2004 PMID: 15452205

26. Arrighi J-F, Pion M, Garcia E, Escola J-M, van Kooyk Y, Geijtenbeek TB, et al. DC-SIGN-mediated infectious synapse formation enhances X4 HIV-1 transmission from dendritic cells to T cells. J Exp Med. 2004; 200: 1279–1288. doi: 10.1084/jem.20041356 PMID: 15545354

27. Dull T, Zufferey R, Kelly M, Mandel RJ, Nguyen M, Trono D, et al. A third-generation lentivirus vector with a conditional packaging system. J Virol. 1998; 72: 8463–8471. PMID: 9756382

28. Tersmette M, Winkel IN, Groenink M, Gruters RA, Spence RP, Saman E, et al. Detection and subtyping of HIV-1 isolates with a panel of characterized monoclonal antibodies to HIV p24 gag. Virology. 1989; 171: 149–155. PMID: 2472701

29. Der SD, Zhou A, Williams BRG, Silverman RH. Identification of genes differentially regulated by interferon α, β, or γ using oligonucleotide arrays. PNAS. 1998; 95: 15623–15628. doi: 10.1073/pnas.95.26.15623 PMID: 9861020
30. Alkhattib G, Combadiere C, Broder CC, Feng Y, Kennedy PE, Murphy PM, et al. CC CKR5: a RANTES, MIP-1alpha, MIP-1beta receptor as a fusion cofactor for macrophage-tropic HIV-1. Science. 1996; 272: 1955–1958. PMID: 8658171

31. Clerzius G, Shaw E, Daher A, Burugu S, Gélinas J-F, Ear T, et al. The PKR activator, PACT, becomes a PKR inhibitor during HIV-1 replication. Retrovirology. 2013; 10: 96. doi: 10.1186/1742-4690-10-96 PMID: 24020926

32. Nie Y, Hammond GL, Yang J-H. Double-stranded RNA deaminase ADAR1 increases host susceptibility to virus infection. J Virol. 2007; 81: 917–923. doi: 10.1128/JVI.01527-06 PMID: 17079286

33. Toth AM, Li Z, Cattaneo R, Samuel CE. RNA-specific adenosine deaminase ADAR1 suppresses measles virus-induced apoptosis and activation of protein kinase PKR. J Biol Chem. 2009; 284: 29350–29356. doi: 10.1074/jbc.M109.045146 PMID: 19710021

34. Okonski KM, Samuel CE. Stress granule formation induced by measles virus is protein kinase PKR dependent and impaired by RNA adenosine deaminase ADAR1. J Virol. 2013; 87: 756–766. doi: 10.1128/JVI.02270-12 PMID: 23115276

35. Cachat A, Alais S, Chevalier SA, Journo C, Fusil F, Dutartre H, et al. ADAR1 enhances HTLV-1 and HTLV-2 replication through inhibition of PKR activity. Retrovirology. 2014; 11: 93. doi: 10.1186/s12977-014-0093-9 PMID: 25389016