Diverse functions for DNA and RNA editing in the immune system

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Biological information is coded in the base sequence of DNA and RNA. It follows that the fidelity of this information is meticulously preserved during its replication, transcription and maintenance, particularly in higher organisms; alterations at the level of genome or transcriptome can have dramatic downstream functional implications. Unintended sequence changes often lead to deleterious consequences. However, despite the pressure to guard against such effects, many biological systems have developed evolved means to harness host editing mechanisms to enhance the genetic diversity required for an effective humoral adaptive immune response (AID). Not surprisingly, some pathogens have targeted mechanisms to alter DNA or RNA sequences and their corresponding information content. Two primary categories of enzymes that edit polynucleotide sequences are the cytidine deaminases (which catalyze deamination of cytidine to uridine) and the adenosine deaminases acting on RNA (ADARs, which catalyze the deamination of adenosine to inosine), function in a variety of host defense mechanisms. These enzymes act in innate and adaptive immune pathways, with both host and pathogen targets. DNA editing by the cytidine deaminase AID mediates immunoglobulin somatic hypermutation and class switch recombination, providing the antibody response with the flexibility and diversity to defend against an almost limitless array of varied and rapidly adapting pathogenic challenges. Other cytidine deaminases (APOBEC3) restrict retroviral infection by editing viral retrogenomes. Adenosine deaminases (ADARs) shape innate immune responses by modifying host transcripts that encode immune effectors and their regulators. Here we review current knowledge of polynucleotide DNA and RNA editors with a focus on these and other functions they serve in the immune system.

The immune system faces a virtually limitless array of pathogens with correspondingly diverse structures and functions. In order to combat potential threats, the host must distinguish “self” from “non-self” targets and direct effector mechanisms accordingly. Innate immune defenses typically recognize common pathogen-associated molecular patterns (PAMPS) and/or molecular elements normally alien to the eukaryotic cell (e.g., viral replication intermediates). The germline-encoded receptors of the innate immune system mediate many key aspects of host defense, but are alone insufficient to defend against all potential pathogens; individually encoded receptors specific to each distinct threat would require a genome of massive size and complexity. Furthermore, pathogens can rapidly adapt to and evade host defenses, necessitating a variable and adjustable response. The adaptive immune system addresses these problems through mechanisms that introduce considerable genetic diversity within the antigen receptor genes of immune cells. In conjunction with cellular selection processes, these processes provide a flexible antigen recognition system while allowing for a host genome of reasonable size. Pathogens employ many strategies to subvert host functions, which demand similarly diverse host defenses. As instruments of informational diversity, polynucleotide DNA and RNA sequence editors are important players in innate and adaptive immunity. In particular, the AID/APOBEC family of cytidine deaminases and ADAR family of adenosine deaminases mediate various immune functions. Their targets include the genomes and transcriptomes of both host and pathogen. Host-directed mRNA editing shapes innate immunity by altering the content of transcripts coding for effector proteins (ADARs). Editing directed at retroviral sequences represents a potent innate defense that can mutate and thereby cripple viral genomes (APOBEC3). Meanwhile, DNA editing at host immunoglobulin (Ig) loci is critical in establishing the genetic diversity required for an effective humoral adaptive immune response (AID). Not surprisingly, some pathogens have evolved means to harness host editing mechanisms to enhance their own functions (ADARs). Here we review current knowledge of polynucleotide DNA and RNA editors with a focus on these and other functions they serve in the immune system.

AID/APOBEC Cytidine Deaminases in the Immune System

APOBEC1, an RNA-editing cytidine deaminase. The first mammalian polynucleotide cytidine deaminase identified,1,2
Apolipoprotein B mRNA editing enzyme, catalytic polypeptide 1 (APOBEC1) is an mRNA editing enzyme with a well-characterized function in lipid absorption. It mediates the tissue-specific and splicing-independent regulation of apolipoprotein B (apoB) isoforms. The full-length apoB-100 isoform is a principal component of hepatic low-density lipoprotein (LDL) particles, while the truncated apoB-48 isoform is predominant in the chylomicrons generated in the small intestine. Transcription and splicing of the apoB mRNA is identical in both tissues. However, in the small intestine, APOBEC1 catalyzes the site-specific deamination of C6666, effectively converting a CAA glutamine codon to a UAA stop codon. Upon translation, apoB-48 is produced and subsequently incorporated into chylomicrons with absorbed dietary lipid.

ApoB editing requires APOBEC1 complementation factor (ACF), an RNA binding protein and component of the APOBEC1 “editosome”. ACF recognizes and binds to an 11 nt “mooring sequence” downstream (3’) of the edited C, and is thought to position the edited base appropriately in the chylomicrons. ACF also functions to suppress nonsense-mediated decay of the edited apoB transcript.

At present, apoB represents the only known physiological APOBEC1 mRNA editing target in healthy tissue. However, there is some evidence that APOBEC1 may also function in the immune system. APOBEC1 associates with and stabilizes several AU-rich mRNAs, thereby influencing their translation independently of editing activity. One such mRNA binding substrate, COX-2, is a key factor in prostaglandin-mediated inflammation. Furthermore, though APOBEC1 expression was long considered exclusive to the intestine, some evidence indicates the presence of APOBEC1 transcripts in mouse secondary lymphoid tissues and several immune cell types (Rosenberg BR and Papavasiliou NF, unpublished data). Further study of potential APOBEC1 targets in intestine and/or leukocytes may reveal novel functions in the immune system.

**Activation-induced cytidine deaminase drives antibody diversification.** Though APOBEC1 was identified because of its function in lipid absorption, the discovery of Activation-induced cytidine deaminase (AID) demonstrated a fundamental role for polynucleotide cytidine deaminases in the immune system.

AID is a central mediator of the adaptive immune response, driving antibody diversification in response to antigenic challenge through two distinct processes: somatic hypermutation (SHM) and class switch recombination (CSR). In SHM, point mutations are introduced within the V(D)J regions of rearranged Ig loci, thereby altering the antigen binding properties of the encoded Ig. SHM occurs in germline center B cells and those B cells that acquire improved antigen binding affinity are positively selected for survival. During this “affinity maturation,” the Ig variable regions accumulate additional mutations and the activated B cells are thereby optimized for a highly specific and potent adaptive immune response. As these modifications are fixed in somatic B cell genomes, they are maintained in clonal expansion and subsequent memory responses. In CSR, a region specific recombination reaction replaces the primary constant (Cμ) region with a downstream constant region (Cγ, Cε, or Cα). Constant regions code for the Fc portions of antibody molecules; recombination between these regions changes the isotype and endows the antibody protein with different effector properties. This genomic and functional flexibility allows for an antigen-appropriate response in many different infection contexts. AID is necessary for both of these processes.

AID was identified in a subtractive hybridization screen comparing resting B cells and B cells stimulated to undergo CSR. Initial sequence analysis of AID revealed that it contains a cytidine deaminase domain with considerable homology to the previously characterized RNA editor, APOBEC1. As such, it was initially hypothesized that AID also edited mRNA. To account for the involvement of AID in both SHM and CSR, it was thought that AID might target two distinct mRNAs, such as a transcript encoding a DNA mutator for SHM and a transcript encoding a region-specific recombinase for CSR. Alternatively, AID might edit a single mRNA encoding a protein that functions in both SHM and CSR. Either mechanism would require task-specific and/or targeting cofactors, similar to the APOBEC1/ACF model. This proposal is referred to as the “RNA editing hypothesis.”

In the alternative “DNA editing hypothesis,” AID acts on and mutates DNA directly. This model fits with earlier theories predicting the existence of a mutating factor that directly targets the Ig locus. Additional characterization demonstrated that ectopically expressed AID could mutate the genomes of several mammalian cell types, yeast, and bacteria. It is unlikely that AID would edit the same mRNA in both prokaryotic and eukaryotic cells in order to code for a novel DNA mutator.

A variety of DNA repair mechanisms were also shown to contribute to both SHM and CSR, further supporting the editing model in which AID acts directly on the genome. As a cytidine deaminase, AID activity was predicted to introduce U:G mismatches in DNA. Such mismatches are typically resolved by Uracil DNA glycosylase (UNG), the primary effector of uracil base excision repair. Interestingly, UNG-deficient animals acquire Ig locus mutations at rates comparable to wild-type controls. However, the spectra of mutations in the absence of UNG are dramatically different. While mutation patterns at A and T bases are similar, mutations at G and C residues are strongly biased towards G to A and C to T events in UNG−/− mice, thereby implicating genomic uridines and their repair in shaping the SHM profile. Aside from SHM, UNG (in combination with the mismatch repair enzyme Msh2) is also necessary for CSR.

Though beyond the scope of this review, numerous additional studies of DNA damage and repair mechanisms in the context of CSR and/or SHM further demonstrated that AID acts directly on genomic cytidines. In SHM, AID deaminates cytidines within rearranged Ig V(D)J segments (Fig. 1A). The recognition and error-prone resolution of these deoxyuridines by the cellular repair machinery leads to the transition and transversion point mutations of B cell affinity maturation. In CSR, AID deaminates cytidines within repetitive sequences between the variable and constant regions of Ig gene segments (Fig. 1B). This editing event (and corresponding DNA breaks) initiates the region-specific
AID-mediated chromosomal translocations have been implicated in the oncogenesis of B cell lymphomas. However, AID may also contribute to tumorigenesis in non-lymphoid tissues. Studies from several groups have shown that AID expression is not restricted to germinal center B cells. AID is expressed in bone marrow pre/pro B cells after infection with a transforming virus, in breast and ovarian tissue upon estrogen stimulation, in prostate cells upon androgen receptor signaling, and in a variety of human malignancies. Though it remains unclear how expression is induced in each case, it seems that AID may affect tumor pathogenesis and development. Recent data indicate that AID can participate in the epigenetic reprogramming of cells to undifferentiated states. Other work has shown that AID activity can contribute to chemotherapeutic resistance and tumor relapse. Oncogenic events represent an infrequent but deleterious outcome of dysregulated and/or mistargeted DNA editing. In the case of AID, the benefit of antibody diversification to host defense generally outweighs these consequences of off-target mutation.

APOBEC3G restricts HIV infection. Detailed functional characterization of APOBEC1 and AID provided a framework for recombination reaction that results in Ig constant region isotype switch. Despite homology to APOBEC1, the majority of available data supports this model of AID as a DNA editor. In this capacity, it plays a central role in adaptive immunity by generating the antibody diversity necessary for host defense against an innumerable variety of antigenic challenges.

**AID and cellular transformation.** Despite benefits in host defense, genomic DNA editing carries significant risks. In the context of CSR, it is thought that AID deamination (together with UNG activity) generates coupled DNA breaks at the Ig locus. Most of these are appropriately repaired, leading to deletional recombination. However, occasionally the breaks may be erroneously resolved, giving rise to chromosomal translocations. The partner loci for such rearrangements can be either genomic sites that can serve as infrequent off-site targets for AID (including many proto-oncogenes), or hypersensitive sites susceptible due to structure (e.g., cruciform DNA at the bcl-2 locus) or function (e.g., enhancer elements). Incorrect break resolution can be caused by deficiencies of DNA repair factors or lack of Ig locus CSR targeting elements.

![Figure 1. AID drives antibody diversity through two distinct mechanisms. (A) In SHM, AID deaminates cytidines within the variable region of Ig loci. Genomic deoxyuridine residues are then resolved by two pathways. Uridine is read as thymidine by replication machinery, leading to transition mutations. Alternatively, UNG excises the edited base, which is followed by abasic site repair via base-excision repair (BER) and mismatch-repair (MMR) enzymes, leading to transversion mutations. (B) In CSR, AID deaminates cytidines within Ig switch (S) regions, leading to double strand breaks. Recombination replaces the primary constant switch region (C_\text{\mu}) with one of several downstream constant regions (C_\gamma, C_\epsilon or C_\alpha), altering the effector properties of the encoded antibody. In this representation, the primary C_\mu region is replaced with a C_\gamma region, thereby causing a switch from the IgM to the IgG_\gamma isotype.](image-url)
APOBEC3G’s place in the cytidine deaminase family provided clues as to how it might function to restrict Δvif/HIV infection. A torrent of work from various groups demonstrated that APOBEC3G restricts HIV by inducing hypermutation in newly reverse-transcribed viral cDNA (Fig. 2).36,37 In non-permissive cells infected with Δvif/HIV, APOBEC3G associates with viral RNA38,39 and/or the nucleocapsid domain of Gag,40 which package the enzyme into newly assembled virions. Nascent viral particles then carry and deliver APOBEC3G to newly infected cells, in which the cytidine deaminase remains associated with the retroviral replication machinery. Upon reverse transcription and following activation by retroviral RNAseH activity,41 APOBEC3G actively deaminates along the retroviral (-)-strand CDNA, introducing C-U changes through the viral retrogenome. Most hypermutated (-)-strand cDNAs do not proceed to second-strand synthesis, though the mechanism for their degradation remains unclear. Those retrotranscripts that do progress to integration carry a heavy mutation burden that drastically compromises the information content of the retroviral genome and prevents the production of functional virus.

Both CD4+ T cells and macrophages, the principle targets of HIV infection in vivo, express high levels of APOBEC3G.42 HIV establishes productive infections in these cell types through the action of Vif, which excludes APOBEC3G from packaging virions primarily by mediating its degradation.43-47 In the infected cell, Vif binds APOBEC3G and recruits a cullin5-elongin B/C-Rbx ubiquitin ligase complex.48,49 APOBEC3G is thereby targeted for ubiquitylation and subsequent degradation in the proteasome. This process allows for the production of virions unfettered by packaged APOBEC3G and corresponding hypermutation. The Vif/APOBEC3G interaction is precise and species-specific; HIV Vif inhibits APOBEC3G from human but not African Green Monkey (AGM), while SIV-AGM Vif inhibits AGM APOBEC3G, but not human.50 This specificity maps to a single amino acid at position 128 in APOBEC3G (Asp in human, Lys in AGM).51-53 Such exacting requirements for association offer a perspective on the powerful selective pressures exhibited by this host-pathogen relationship. In fact, phylogenetic analysis demonstrates that the APOBEC3 subfamily has been under markedly strong positive selection throughout primate evolution.54,55 At present, it appears that the sole function of Vif is to act as a viral countermeasure against APOBEC3G and related family members. The evolutionary pressure to devote such a significant portion of the remarkably efficient HIV genome to this purpose illustrates the considerable impact this gene family can have on host defense.

APOBEC3 family cytidine deaminases have diverse antiviral activities. The antiviral activity of the polynucleotide cytidine deaminases is not limited to APOBEC3G, nor is it directed
solely against HIV. Aside from APOBEC3G, additional family members APOBEC3B and APOBEC3F can also restrict HIV replication (reviewed in ref. 56). Interestingly, APOBEC3 cannot be suppressed by Vif. However, this may not be relevant in vivo, as only APOBEC3G and 3F are expressed by CD4+ T cells and macrophages. Indeed, viral sequence analysis of HIV patient isolates reveals mutation patterns consistent with APOBEC3G and 3F activity.59

Apart from HIV, it seems that most retroviruses are somewhat susceptible to members of the APOBEC3 subfamily; Murine leukemia virus (MLV), Equine infectious anemia virus (EIAV), foamy retroviruses and even hepatitis B virus (HBV) can be restricted by different APOBEC3 deaminases (reviewed in ref. 60). Though not a true retrovirus, HBV replicates its partially double-stranded DNA genome by a reverse transcription mechanism,61 which represents a likely target for these deaminases. However, reverse transcription may not be a requirement for susceptibility to all APOBEC3 subfamily members; APOBEC3A can dramatically inhibit replication of adeno-associated virus (AAV), a small single-stranded DNA parvovirus that replicates via the host cell polymerase machinery.62 It remains to be seen if AAV represents a unique exception or if APOBEC3 subfamily members exhibit broad antiviral activity against diverse non-retroviruses.

**Endogenous retroelements are suppressed by APOBEC3 family cytidine deaminases.** The suppressive activity of the APOBEC3 proteins is not limited to exogenous retroviruses; several family members have also been demonstrated to restrict a variety of endogeneous retroelements. Such host-encoded retroelements constitute significant portions of mammalian genomes and include the long terminal repeat (LTR)-containing endogenous retroviruses (ERVs), as well as non-LTR sequences such as long interspersed nuclear element-1 (LINE-1). Many of the APOBEC3 deaminases can suppress retrotransposition of ERVs63 and (LINE-1) elements.64 Though it remains unclear if the precise mechanism by which APOBEC3 proteins inhibit endogenous retroelements is identical to that employed in exogenous viral stimulation, both appear to be significant targets for this defense system.

As the existence of functionally active ERVs in humans remains uncertain, the physiological relevance of APOBEC3 subfamily activity against these host-encoded sequences in vivo is not immediately apparent. Perhaps retroelement inhibition represents an “original” function of the APOBEC3 proteins; activity against exogenous lentiviruses may have been co-opted later in the evolutionary history of this gene family. Indeed, despite dramatic rates of positive selection throughout primate evolution, the apparent selective pressures on the APOBEC3 genes predate the emergence of modern primate lentiviruses by millions of years.54,55 In addition, the evolutional expansion of the APOBEC3 family correlates with a dramatic decrease of endogenous retroelement activity in primate genomes as compared to rodents.65 Thus, the APOBEC3 proteins may represent an ancient defense system for protecting genome integrity, be it from actively mobile endogenous retroelements or the primitive retroviruses from which they were derived.

**Adenosine Deaminases Acting on RNA (ADARs) in the Immune System**

ADARs bind and edit dsRNA. The adenosine deaminases acting on RNA (ADARs) bind to dsRNA structures and catalyze the conversion of adenosine to inosine (reviewed in refs. 66–69). Inosine is read as guanosine by translational and reverse transcriptional machinery. Thus, ADARs effectively induce A to G changes and thereby increase transcriptome diversity. A to I editing is the most common editing event in higher eukaryotes and has been recently recognized to occur predominantly in RNA duplexes formed from inverted Alu or LINE repeats in the untranslated regions (UTRs) of primary mRNA transcripts.70–72 The biological consequences of editing within these sequence repeats are still uncertain but may serve a role in gene regulation. In fact, dsRNA Alu pairs in 3’UTRs can suppress gene expression, an event which correlates with A to I editing and the nuclear retention of certain transcripts.73

The ADARs were first identified as dsRNA-unwinding enzymes in *Xenopus laevis*74,75 but were redefined shortly thereafter as dsRNA-editing enzymes.76,77 Three ADARs (ADAR1-3) have been identified, based on conservation in the C-terminal deaminase and double-stranded RNA-binding domains.69 ADAR1 and ADAR2 demonstrate deaminase activity78–80 but the function of ADAR3 remains elusive.81,82 ADAR1 is the best-characterized member of this gene family. Specifically, two differentially localized isoforms of ADAR1 have been identified. The larger form, ADAR1-L or ADAR p150, is present in both the cytosol and nucleus and is regulated by an interferon-inducible promoter.84–86 ADAR1-S or ADAR1 p110, the smaller form, is exclusive to the nucleus and driven by two constitutively active promoters.87

ADARs are highly expressed in brain tissue and are important for neurological function. Site-specific ADAR editing in the brain, notably in glutamate and serotonin receptor mRNAs, leads to single codon changes and thereby protein products with altered physiological functions (reviewed in refs. 66, 67 and 69). While many of these neurological targets are reasonably well characterized, roles for ADARs in the immune system are presently under extensive investigation. In particular, ADAR1 has been implicated in hematopoiesis, viral immunity and regulation of the interferon response.

**ADAR1 is essential for immune cell development.** Multiple lines of evidence demonstrate the importance of A to I mRNA editing in the immune system, starting with a requirement for ADAR1 in immune cell development. More specifically, knockout studies have defined a role for ADAR1 in fetal hematopoiesis. ADAR1-deficient mice are known to suffer from liver and bone marrow hematopoietic defects and typically die at embryonic day 11.5–12.5.88 Using ADAR1 conditional knockout models, Orkin and colleagues have further demonstrated that ADAR1 is necessary for the maintenance of both fetal liver-derived and adult bone marrow hematopoietic stem cells (HSCs).89 The authors propose that ADAR1 maintains HSC populations by protecting cells from early apoptotic events, possibly by regulating interferon signaling pathways (see below). Other recent work, however, suggests that ADAR1 activity is essential in the
differentiation of hematopoietic progenitor cells (HPCs), rather than HSCs.\(^{39}\) Although the question remains as to which stage of hematopoiesis requires ADAR1, it is clear ADAR1-deficient precursors do not develop into mature immune cells.

**ADARs can act as antiviral enzymes.** As an interferon-inducible gene, it is not surprising that ADAR1 has been implicated in antiviral defense. As described above, it appears to be a critical regulator of the interferon response, which implies activity on host RNA targets. However, as an RNA editing enzyme, ADAR1 could presumably edit viral RNAs in a process not unlike APOBEC3 hypermutation of retroviral cDNA. Indeed, like HIV antagonism of APOBEC3G by the Vif protein, vaccinia virus and adenovirus have evolved ADAR1 inhibitors that specifically impair ADAR1 deaminase activity,\(^{91,92}\) suggesting an anti-viral function for ADAR1. ADAR1 does edit a broad spectrum of viral targets but paradoxically seems to play both pro- and anti-viral roles in infection.

A to I editing has been observed in diverse viral RNAs, including influenza virus,\(^{93}\) parainfluenza virus,\(^{94}\) lymphocytic choriomeningitis virus (LCMV),\(^{95}\) vesicular stomatitis virus (VSV),\(^{96}\) measles virus,\(^{106-108}\) polyomavirus,\(^{97}\) hepatitis D virus (HDV),\(^{98}\) and hepatitis C virus (HCV).\(^{99}\) Despite an early recognition of A to I biased hyperediting in viral transcripts during persistent and lytic infections,\(^{100}\) the function and consequences of many of these editing events is still under investigation. A clear example of direct ADAR antiviral editing has been observed in LCMV RNA transcripts.\(^{97}\) In vitro and in vivo studies of LCMV infection demonstrated high rates of ADAR1-specific A-to-I mutations, leading to dysfunctional glycoproteins and impaired viral infectivity. In addition, recent work on HCV infection identified an ADAR1 editing-dependent loss of HCV replicons.\(^{99}\) Replicon loss was thought to be attributable to an inosine-specific RNase\(^{101,102}\) or to viral genome instability introduced by weakly base-pairing inosine nucleotides.\(^{99}\) Another study using a polyoma virus system suggests that promiscuous A to I editing of transcripts in the nucleus may recruit an inosine-specific RNA binding protein, p54nrb. Along with the splicing factor PSF and the matrix structural protein matrin 3, binding of p54nrb functions to retain edited transcripts in the nucleus, preventing export and translation.\(^{75,103}\) The authors suggest that this mechanism regulates the export of early stage transcripts, though it is possible that retention functions to sequester viral transcripts, inhibiting translation of essential viral proteins.

ADAR family members can also directly restrict viral replication independent of its editing function. ADAR1 associates with and activates transcription factors involved in anti-viral gene expression, including nuclear factor 90 (NF90).\(^{104}\) ADAR1 interacts with NF90 via an undefined dsRNA bridge and leads to the upregulation of NF90-regulated genes, including IFNβ.

**ADARs can support viral infection.** In apparent contradiction to several well-characterized anti-viral functions, ADAR proteins can also promote viral infection and replication.

While p110 ADAR1 is the predominant isoform during embryogenesis,\(^{105}\) the p150 interferon-inducible form is more prevalent in hematopoietic stem cells of the adult.\(^{69}\) Expanding on their work with the conditional knockout mice, Orkin and colleagues showed that ADAR1 acts as novel suppressor of the Type I interferon response. Specifically, gene signatures of uninfected ADAR1-deficient HSCs and erythroid precursors are highly similar to those of virus-infected or interferon treated cells. Additionally, ADAR1 knockout embryos were found to have significantly higher levels of type 1 interferon in extracellular fluid. It remains to be explained how ADAR1 dampens the interferon response in the absence of viral infection. ADAR1 could be editing a microRNA molecule or target, neutralizing an unidentified immunostimulatory dsRNA or functioning in the regulation of interferon-induced gene expression. A compelling argument is that ADAR1 functions as a novel cytosolic dsRNA binding protein competing for substrates with the DNA-dependent activator of interferon regulatory factors (DAI), a cellular dsRNA sensor. In sequestering immunostimulatory dsRNA (e.g., viral replication intermediates) from DAI, ADAR1 may impair downstream innate immune signaling.\(^{106}\) The absence of ADAR1 dsRNA binding activity could lead to aberrant activation of the innate immune response and a corresponding induction of interferon production.

Perhaps related to its role in regulation of the interferon response, ADAR1 has been observed to bind and impair host antiviral response elements. Work on measles viral infection has shown ADAR1 inhibition of protein kinase regulated by RNA (PKR) and interferon regulatory transcription factor-3 (IRF-3).\(^{107}\) Other groups have observed similar ADAR1 regulation of PKR during VSV\(^{108,109}\) and HIV (see below) infections as well. Inhibition of these proteins compromises the host cell ability to respond to viral signals, potentially promoting persistent infection. Interestingly, hyperediting of viral transcripts has been observed in patients suffering from a complication of persistent measles infection. This paradoxical situation, in which there is evidence of ADAR1 activity both supporting and antagonizing viral infection suggests a nuanced role for ADAR1 in host-virus interaction.

It appears that ADAR1 can dampen type I interferon signaling by several mechanisms. This function may have evolved to protect the host from an inappropriate (i.e., in uninfected cells) or overactive (i.e., disproportionate response to infection) interferon response. This regulation might serve to counterbalance the effector functions of ADAR1 as an antiviral enzyme. However, it seems that in certain infections, ADAR1 anti-viral activity is not only impaired but the enzyme itself may be co-opted by viruses to support infection. In this context, the significance of ADAR1 hyperediting viral RNA remains unclear. Is it merely an inert footprint of ADAR1 activity or do some editing events still function to restrict (or promote) viral replication? Additional studies, particularly using the recently engineered conditional ADAR1-deficient mouse will likely address these problems.

**ADAR1 acts in the host response to HIV infection.** Like its deaminase cousin APOBEC3G, ADAR1 has recently been found to target and edit HIV-1 sequences. However, unlike APOBEC3G, which edits retrotranscript cDNA and restricts viral infection, ADAR1 targets viral RNA and enhances HIV protein expression, replication and infectivity.\(^{110}\) These effects are mediated by both editing-dependent and editing-independent
mechanisms. Overexpression of ADAR1 in HIV-1 producer cells dramatically enhances expression of several viral proteins irrespective of RNA editing, possibly due to ADAR inhibition of PKR. Active RNA editing may also regulate virus production as ADAR1, but not experimentally-engineered catalytic mutants, increased the release of progeny virions 2-fold and enhanced HIV-1 infectivity 2.5-fold.

The physiological significance of ADAR1 action on the HIV-1 life cycle is still unclear, as are the exact viral sequence targets for ADAR1 editing. One analysis found that a majority of A to G changes occur in a region of the 5’UTR shared by all HIV-1 transcripts. A different study identified a specific ADAR1 editing site in the HIV env gene. Further investigation is needed to understand the regulation, impact and mechanism of ADAR1 editing in HIV-1 RNA. Of particular interest are the opposing roles of the cytidine (APOBEC3G) and adenosine (ADAR1) deaminases in HIV-1 infection. While APOBEC3G targets and edits virion RNA to restrict and inhibit HIV-1, ADAR1 appears to be harnessed by the virus itself to enhance viral protein expression, infectivity and virion release.

**ADAR1 edits hepatitis D viral RNA.** The best characterized ADAR-mediated viral editing event occurs during HDV infection. HDV is a subviral pathogen that is dependent on a concurrent infection with HBV; it requires HBV surface antigen to infect hepatocytes. The genome of the HDV virus is an ideal substrate for ADARs, as its single-stranded negative sense circular RNA forms secondary structures with frequent duplex regions. In addition to co-opting the hepatitis B surface antigen, the HDV genome encodes its own HDV-specific surface antigen (HDVAg), the antigenome transcript of which is edited by ADAR1 in a site-specific manner. The HDVAg occurs in two forms, both essential for the viral life cycle. The short form, HDVAg-S, is required for viral RNA replication while the long form (HDVAg-L) directs viral genome assembly and packaging. ADAR1 targets the HDV antigenome at a specific “amber/W site,” thereby converting a stop codon (UAG) to a tryptophan (UIG). This allows for the translation of the long form of the HDV surface antigen. The HDVAg-L then restricts viral replication in a trans-dominant fashion by binding HDAg-S and interrupting HDAg-S homodimers.

In unstimulated cells, amber/W site editing occurs via the ADAR1-S isoform and serves to support viral assembly; HDVAg-L halts replication and mediates viral packaging by binding clathrin heavy chain. More recent studies have shown, however, that ADAR1 can serve an anti-viral role when editing in a more promiscuous fashion. Overexpression of ADAR1 or ADAR2 (though ADAR2 is not induced in natural infection) leads to hyperediting at non-amber/W sites, producing higher levels of HDAg-L as well as other HDAg mutants that can also bind HDAg-S and inhibit replication. In an interferon-stimulated system, which more closely mimics both early natural infection and IFN-treated infection, the large form of ADAR1 is highly expressed, is the predominant editor, and increases editing 2-fold. It is unclear, however, whether IFN stimulation would induce high enough levels of ADAR1-L to edit promiscuously or to significantly impair viral replication. In fact, while a replication-competent mutant virus with enhanced editing at the amber/W site displays increased levels of HDAg-L early in infection and impaired replication at a later time point, editing and replication seem to be coupled. Amber/W site editing ceases with replication, indicating a natural feedback mechanism controlling aberrant editing by elevated ADAR1 activity.

**Closing Remarks**

Polynucleotide deaminases function as drivers for diversity by altering the information content of RNA and DNA sequences. Here, we have described some of the processes by which this family of enzymes function in the host response to pathogenic challenges. The polynucleotide deaminases act on a variety of molecular substrates (including ssRNA, dsRNA and ssDNA), catalyze different editing events (C to U; A to I), edit a diverse set of sequence targets, and not surprisingly, play a number of roles in innate and adaptive immunity. Significantly, the immune functions of these editing enzymes manifest on both sides of the host: pathogen interface. ADAR1 drives the plasticity of the Ig loci and thereby mediates key features of the adaptive immune response. APOBEC3 and ADAR proteins are potent innate immune effectors and can incapacitate viral genomes by directing their mutagenic activity against viral sequences. Paradoxically, ADAR1 can also promote viral replication and/or diversification by acting on both pathogen and host targets. Though the past decade of research has vastly increased our understanding of these enzyme families, especially with regard to immune function, many unanswered questions remain. In particular, there are likely roles for RNA/DNA editing in additional host defense mechanisms. The identification and functional characterization of novel APOBEC and ADAR targets may reveal important novel functions for these enzymes in the immune system.

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References


