Cell, Vol. 113, 803–809, June 13, 2003, Copyright ©2003 by Cell Press

DNA Deamination Mediates Innate Immunity to Retroviral Infection

Reuben S. Harris,[1,3,4,*] Kate N. Bishop,[2,3]
Ann M. Sheehy,[2] Heather M. Craig,[2]
Svend K. Petersen-Mahrt,[1] Ian N. Watt,[1]
Michael S. Neuberger,[1] and Michael H. Malim[2]
[1]Medical Research Council Laboratory of Molecular Biology
Hills Road
Cambridge CB2 2QH
United Kingdom
[2]Department of Infectious Diseases
Guy's, King's and Street Thomas' School of Medicine
King's College London
London SE1 9RT
United Kingdom

Summary

CEM15/APOBEC3G is a cellular protein required for resistance to infection by virion infectivity factor (Vif)-deficient human immunodeficiency virus (HIV). Here, using a murine leukemia virus (MLV)-based system, we provide evidence that CEM15/APOBEC3G is a DNA deaminase that is incorporated into virions during viral production and subsequently triggers massive deamination of deoxycytidine to deoxyuridine within the retroviral minus (first)-strand cDNA, thus providing a probable trigger for viral destruction. Furthermore, HIV Vif can protect MLV from this CEM15/APOBEC3G-dependent restriction. These findings imply that targeted DNA deamination is a major strategy of innate immunity to retroviruses and likely also contributes to the sequence variation observed in many viruses (including HIV).

Introduction

Productive infection by HIV depends on the virus-encoded protein Vif (Fisher et al., 1987; Strebel et al., 1987). Vif expression is necessary only in the virus-producing cell; in its absence, infection of a subsequent target cell terminates at a postentry step through the action of an innate antiviral mechanism (Sova and Volsky, 1993; von Schwedler et al., 1993; Simon and Malim, 1996; Madani and Kabat, 1998; Simon et al., 1998a). How Vif mediates HIV infectivity is not yet known and few clues are apparent in its amino acid sequence as it is highly variable amongst HIV isolates and shows little similarity to other proteins. But, central to this infection restriction mechanism is the human protein CEM15/APOBEC3G, which is expressed in human T cells (the major targets for HIV infection in vivo) and has been shown to be a potent inhibitor of Vif-deficient HIV (Sheehy et al., 2002).

CEM15/APOBEC3G is a member of the APOBEC family of proteins of which the RNA editing protein APOBEC1 is the founder (Teng et al., 1993; Jarmuz et al., 2002). Similarity to APOBEC1 suggested that CEM15/APOBEC3G might also function by deaminating cytosine in RNA—either a host message or the viral RNA genome—although it has not been demonstrated to have any RNA editing activity.

However, CEM15/APOBEC3G has been shown to be able to act as a DNA mutator in *E. coli*, with the mutations attributable to dC→dU deamination (Harris et al., 2002). This led us to speculate (Harris et al., 2003) that CEM15/APOBEC3G triggers the block to retroviral infectivity by deaminating dC→dU in the first (minus)-strand of the viral cDNA during a postentry step of its lifecycle. Here, this hypothesis is tested using a helper virus-dependent MLV-based assay system. We show that MLV produced using cells expressing CEM15/APOBEC3G has a diminished infectivity as monitored using fluorescent protein-encoding genes embedded in viral sequence. Moreover, this correlated with the introduction of a phenomenally high mutation load into the viral cDNA, nearly all the mutations being G→A substitutions as read-out on the viral plus (genomic) strand. This result is only consistent with CEM15/APOBEC3G causing massive deamination of dC→dU in the viral minus (first cDNA) strand, thus establishing CEM15/APOBEC3G as the critical component of this innate defense mechanism. Furthermore, HIV Vif expression is able to counteract the CEM15/APOBEC3G effect on MLV infectivity indicating that Vif serves to overcome a general mode of innate antiretroviral defense that is mediated by DNA deamination.

Results

CEM15/APOBEC3G Can Deaminate DNA

We first used a biochemical assay to assess whether CEM15/APOBEC3G was directly capable of deaminating DNA in vitro. Purified, recombinant CEM15/APOBEC3G was indeed able to deaminate dC→dU in a single-strand DNA oligonucleotide substrate (Figure 1), an activity that has recently also been observed with other APOBEC family members, AID (Bransteitter et al., 2003; Chaudhuri et al., 2003) and APOBEC1 (Figure 1A, lane 1 and Petersen-Mahrt and Neuberger, 2003).

MLV Produced in CEM15/APOBEC3G$^+$ Cells Has Diminished Infectivity

In addition to regulating the infectivity of lentiviruses such as HIV, earlier work suggested that Vif also stimulates infection and replication of the distantly related oncoretrovirus MLV (Simon et al., 1998b). This raised the possibility that CEM15/APOBEC3G might also be able to impede infection by MLV. We therefore sought to develop a helper virus-dependent, MLV-based assay system. Stocks of recombinant MLV encoding yellow

*Correspondence: rsharris@mrc-lmb.cam.ac.uk
[3]These authors contributed equally to this work.
[4]Present Address (beginning August 1, 2003): Department of Biochemistry, Molecular Biology and Biophysics, University of Minnesota, 6-155 Jackson Hall, 321 Church Street SE, Minneapolis, MN 55455.

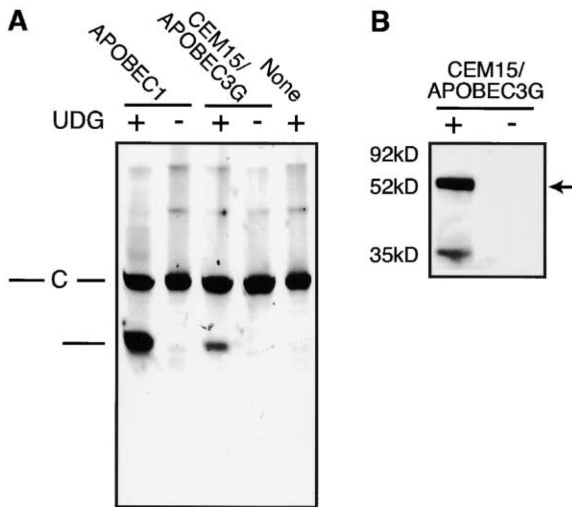


Figure 1. DNA Deamination by CEM15/APOBEC3G

(A) Deamination of dC within a single-stranded oligonucleotide by purified His-tagged CEM15/APOBEC3G (as well as by recombinant APOBEC1 control) was monitored by subsequent treatment of the oligonucleotide with uracil-DNA glycosylase (UDG)/NaOH which breaks the oligonucleotide at the site of dC→dU deamination (illustrated to the left of the gel image).

(B) Western blot of *E.coli* extracts expressing recombinant CEM15/APOBEC3G (upper band, lane 1) or a control plasmid (lane 2).

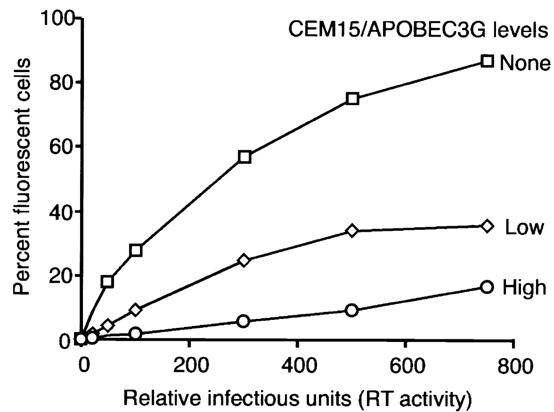


Figure 2. CEM15/APOBEC3G Diminishes MLV Infectivity

293T-derived stocks of YFP-encoding MLV produced by cotransfection with a control vector or with 1 μg or 3 μg of pCEM15:HA were used in challenges of *Mus dunni* fibroblasts across a range of input inocula (normalized units of RT). Similar data were obtained in parallel challenges of N-3T3 cells (data not shown).

fluorescent protein (YFP) were produced in 293T cells (a line not expressing CEM15/APOBEC3G; Sheehy et al., 2002) in the presence or absence of transfected CEM15/APOBEC3G. A range of viral inocula as measured by reverse transcriptase (RT) activity was then used to challenge murine fibroblasts, and productive infection was monitored ~48 hr later by flow cytometric analysis. As seen in Figure 2, the presence of CEM15/APOBEC3G had a dose-dependent inhibitory effect on MLV infection that was clearly apparent at all levels of input inocula (e.g., for inocula that contained 300 relative units of RT, the presence of CEM15/APOBEC3G during virus production caused about a 10-fold reduction of infectivity). Thus, the antiretroviral property of CEM15/APOBEC3G can be extended to the simple (non-*vif* encoding) retrovirus, MLV.

G→A Mutation of MLV DNA Replication Intermediates Following Virus Production on CEM15/APOBEC3G$^+$ Cells

To begin to assess whether retroviral cDNA might be the physiological substrate for the DNA deaminating activity of CEM15/APOBEC3G, a GFP-encoding MLV was produced in 293T cells stably expressing (or not expressing) human CEM15/APOBEC3G. As in the YFP experiments, expression of CEM15/APOBEC3G in the producer cell resulted in a substantial inhibitory effect on MLV infection, as judged by diminished fluorescence of the reporter cells (Figure 3A).

Reasoning that dC→dU deamination of the retroviral reverse transcripts (cDNA) could give rise to inactivating mutations of the GFP gene, we speculated that some of the GFP-negative target cells could nevertheless have been infected but with the GFP gene having been rendered nonfunctional by CEM15/APOBEC3G-induced mutations. Target cells that were negative (-lo) for GFP expression (as well as their GFP-hi counterparts) were therefore isolated by flow cytometry and the retroviral DNA present in cell lysates amplified by PCR, cloned, and sequenced.

A comparison of the GFP gene sequences recovered from GFP-lo cells revealed a striking level of plus-strand G→A transition mutation, but only when the input virus was derived from CEM15/APOBEC3G-expressing cells (Figures 3B and 3C; Figure 4). A total of 435 G→A transitions were found distributed over 86 dG residues in 38 sequences (27,740 bp) yielding a mutation frequency of >1.5 mutations per 100 bases. In contrast, only three separate G→A transitions were found in 47 sequences (34,310 bp) derived from the GFP-lo sorted reporter cells that had been challenged with MLV derived from a non-CEM15/APOBEC3G expressing producer cell line. Both sequence cohorts contained six other base substitution mutations likely attributable to RT or PCR errors, although two deletions were also detected in the G→A transition-littered sequences.

The level of mutation was so high (a median of 11.5 mutations per virus-derived 730-nucleotide GFP gene sequenced) that we anticipated that mutations would be evident even in DNA obtained from GFP-positive target cells. This was indeed the case. A surprising total of 299 G→A transition mutations were found distributed over 47 retroviral cDNAs obtained from the GFP-hi cells that were sorted from the population challenged with MLV-GFP produced from CEM15/APOBEC3G-expressing cells (Figures 3B and 3C; Figure 4). Thus, even with enrichment for the brightest fluorescing cells, 91% (43/47) of the amplified sequences harbored mutations yielding a frequency of 0.9 mutations per 100 bases. Although when compared closely to mutations in sequences derived from GFP-lo cells, the types of mutations apparent in sequences from GFP-hi cells are significantly different (e.g., creating proportionately fewer amino acid changes).

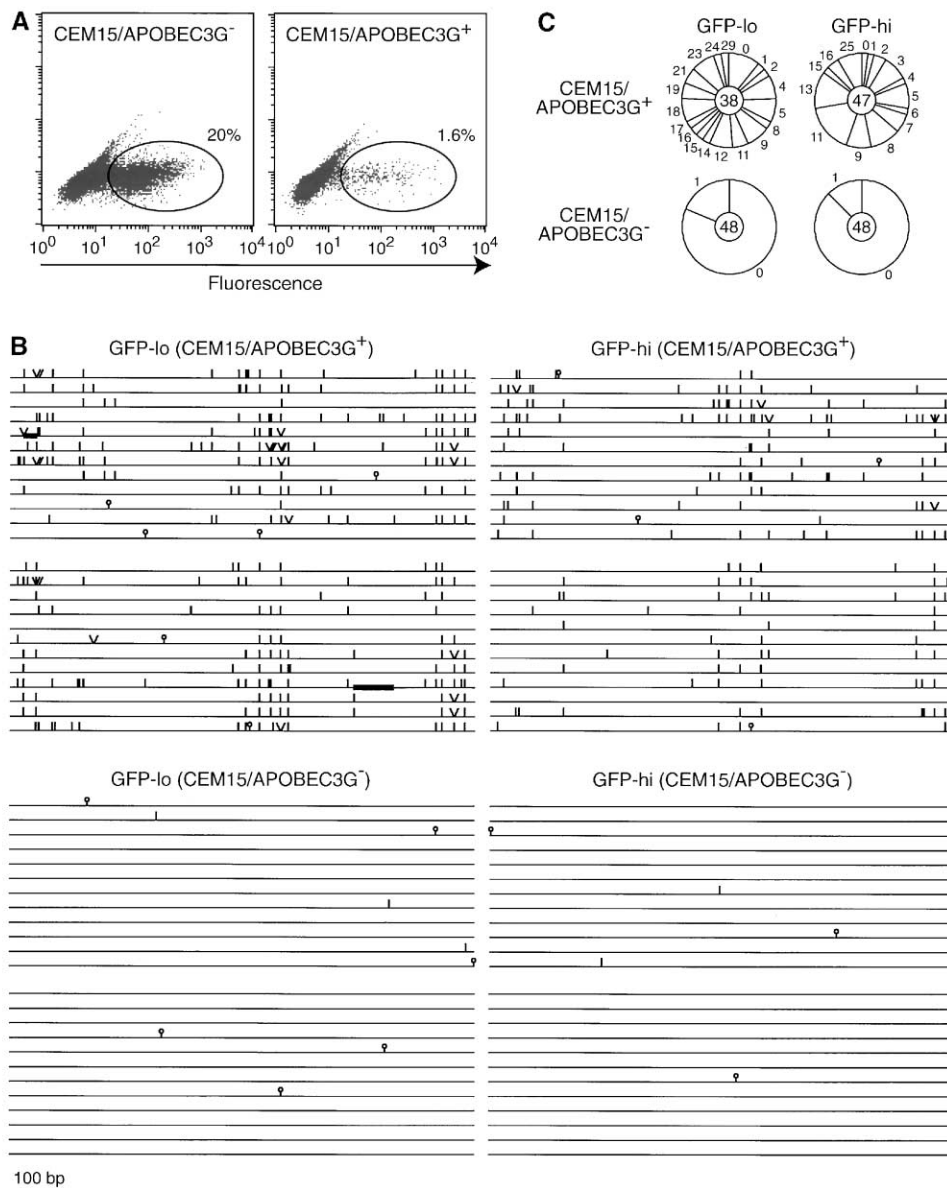


Figure 3. Mutation of Retroviral First-Strand cDNA by CEM15/APOBEC3G

(A) GFP fluorescence of target 293T cells 48h after challenge with equivalent levels of MLV-GFP virions derived from 293T cells stably expressing CEM15/APOBEC3G or a control plasmid. The percentages of cells within the GFP-positive window are indicated.

(B) Profiles of the relative positions of the G→A transition mutations apparent in 12 representative 730 bp GFP sequences derived from GFP-lo and GFP-hi populations of cells challenged with MLV-GFP grown on CEM15/APOBEC3G-expressing cells or nonexpressing controls. Two independently derived CEM15/APOBEC3G$^+$ clones (and two independent controls) were analyzed for each experiment (separated by a space). The G→A transitions are depicted by vertical lines whereas other single nucleotide substitutions (20 in total) are indicated by lollipops and thick, horizontal black bars represent the two deletions detected. Note that each pair of panels (lo and hi) represents sequences recovered from 293T target cells, but the viral stocks used in each experiment (each panel pair) were derived from independent clones either expressing or not expressing CEM15/APOBEC3G.

(C) A comparison of the extent of GFP mutation in the different samples. The pie charts depict the proportion of MLV sequences carrying the indicated number of mutations within the 730 bp interval sequenced. The total number of sequences determined in each data set is indicated in the center.

The mutational onslaught experienced by the GFP gene generates some obviously nonfunctional variants (Figure 4). Although, these were especially apparent in the GFP-lo populations, probable nonfunctional variants were still evident in cDNAs obtained from the GFP-hi population, presumably reflecting multiple infections of individual cells.

The dramatic mutation of retroviral cDNA seen here is strictly dependent on CEM15/APOBEC3G. Mutated GFP genes were rarely obtained from target cells in-

Figure 4. Retroviral cDNA Mutation Spectra

(A) Mutations detected amongst GFP sequences amplified from GFP-lo enriched target cell populations. Mutations derived from MLV-GFP challenged target cells in which the viral stocks used were grown in CEM15/APOBEC3G-expressing cells are depicted above the 730 bp consensus (the viral plus or coding strand is shown) and those derived from vector-expressing cells are shown below. Deletions are boxed. (B) GFP mutations from GFP-hi enriched cell populations.

fected with virus produced in the absence of CEM15/APOBEC3G. Specifically, a total of only 9 and 6 mutations were observed in 47 and 48 sequences from the GFP-lo and -hi sequences respectively, an overall frequency of only 0.012 mutations per 100 bases (Figures 3B and 3C; Figure 4). This background mutation differed from CEM15/APOBEC3G-triggered mutation not just in terms of frequency (more than 50-fold), but also in terms of pattern (not focusing on major hotspots) and base substitution types (less than half are G→A transitions).

The CEM15/APOBEC3G Mutational Bias

When viruses were produced from CEM15/APOBEC3G-expressing cells, virtually all the mutations (734 out of 744) were G→A transitions as judged on the viral plus-strand (Figure 5A). Since both genetic (Harris et al., 2002) and biochemical (Figure 1) evidence indicates that CEM15/APOBEC3G deaminates dC→dU in DNA, the G→A transitions detected in the PCR-amplified GFP genes can only be attributed to CEM15/APOBEC3G-mediated dC→dU deamination in the retroviral minus (first)-strand cDNAs.

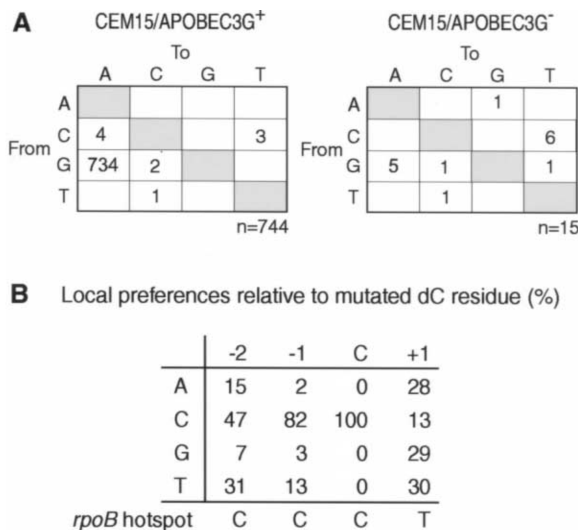
Interestingly, the distribution of mutations along the GFP gene was noticeably nonrandom. The great majority of transition mutations attributable to deamination targeted to dC residues, including those piled up at major hot spots, are preceded by a 5′-dT/dC (pyrimidine)-dC consensus (Figure 5B). This specificity accords well with the context preference that was previously evident from CEM15/APOBEC3G-induced mutation in the *rpoB* gene of *E. coli* (Harris et al., 2002).

CEM15/APOBEC3G Is Incorporated into MLV Virions

The CEM15/APOBEC3G-mediated suppression of HIV infection is thought to be accomplished by protein transferred as a virion component from virus producing cells into target cells (Sheehy et al., 2002). To show that a similar mechanism could apply in the MLV system used here, viral particles were recovered from culture supernatants produced in the presence or absence of CEM15/APOBEC3G and subjected to immunoblot analysis (Figure 6A). As seen in lane 2, CEM15/APOBEC3G is indeed incorporated into MLV virions. This presumably ensures association with reverse transcription complexes in newly infected cells and proximity to nascent cDNA substrates for the execution of dC deamination.

HIV Vif Can Counteract the CEM15/APOBEC3G-Dependent Diminution of MLV Infectivity

Finally, because CEM15/APOBEC3G-mediated inhibition of HIV infection is counteracted by Vif, we also wished to test whether this held true for MLV. Coexpression of Vif in CEM15/APOBEC3G-expressing cells (either transiently or stably) was able to rescue much of the productive MLV infection as monitored by fluorescence (Figures 6B and 6C).



From \ To	A	C	G	T
A				
C	4			3
G	734	2		
T		1		

CEM15/APOBEC3G+, n=744

From \ To	A	C	G	T
A			1	
C				6
G	5	1		1
T		1		

CEM15/APOBEC3G−, n=15

	-2	-1	C	+1
A	15	2	0	28
C	47	82	100	13
G	7	3	0	29
T	31	13	0	30
rpoB hotspot	C	C	C	T

Figure 5. Nature and Local Preferences of CEM15/APOBEC3G Mutation

(A) Nucleotide substitution preferences for the entire set of GFP mutations detected in target cells infected with MLV-GFP that had been grown on a CEM15/APOBEC3G-expressing producer cells in comparison to those detected in target cells infected with MLV-GFP grown on a vector-expressing producer cells.

(B) Delineation of the preferred local sequence context for CEM15/APOBEC3G-mediated dC deamination in MLV-GFP. All 734 mutated positions were aligned with respect to the dC residue targeted for deamination on the minus (first)-strand cDNA and the frequency (as a percentage) with which each of the four bases is found at adjacent positions was calculated.

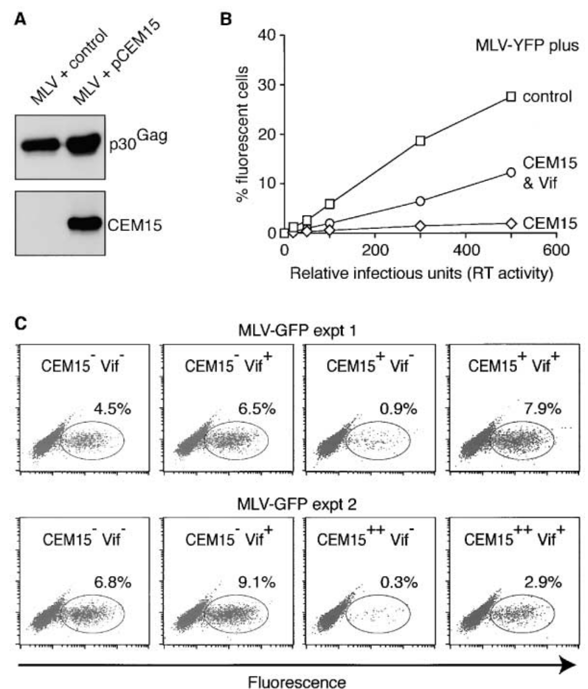


Figure 6. CEM15/APOBEC3G Is Packaged into MLV Particles and HIV Vif Is Able to Inhibit Its Function

(A) YFP-encoding MLV virions were purified from cells (Figure 2) that did not (left lane) or did express CEM15/APOBEC3G (right lane) and analyzed by immunoblotting using antibodies specific for MLV Gag or CEM15/APOBEC3G (HA epitope).

(B) HIV Vif diminishes CEM15/APOBEC3G-mediated immunity to YFP-encoding MLV as monitored using the transient expression system (as in Figure 2) in the presence of pCEM15:HA (3 μg) with or without pcVIF (1 μg).

(C) Restoration of infectivity of CEM15/APOBEC3G-exposed MLV-GFP by expression of HIV Vif during viral stock production from 293T cells stably expressing CEM15/APOBEC3G (as in Figure 3A). The marginal restoration of infectivity in experiment 2 correlates with the approximately 3-fold higher expression of CEM15/APOBEC3G in this cell line over that of the cells used in experiment 1.

Discussion

The results described here indicate that CEM15/APOBEC3G packaged in progeny virions is carried forward into target cells where it acts to deaminate dC→dU in the minus (first)-strand cDNA of the retrovirus.

How might such CEM15/APOBEC3G-mediated DNA deamination of reverse transcripts contribute to innate immunity during normal retroviral infection in vivo? One possibility is that the level of mutation achieved is (as may be the case in the experimental system used here) so high that it jeopardizes viral expansion (Figure 7, left schematic). Indeed, hypermutation has been described in HIV and is typically characterized by excessive G→A transitions (Vartanian et al., 1994; Janini et al., 2001). Moreover, it was shown very recently that this bias is particularly clear in Vif-deficient HIV (Lecossier et al., 2003).

Alternatively, the CEM15/APOBEC3G-mediated blockade of HIV infection has been shown to be accompanied by a failure to accumulate retroviral cDNAs in target cells (Simon and Malim, 1996). This is consistent with the idea that, rather than simply leading to a massive accumulation of viral cDNA mutations, dC→dU deamination of retroviral first strand could trigger ablation of viral infection since the presence of uracil in the first-strand cDNA could recruit components of the base excision repair pathway resulting in the severance of viral replication intermediates (Figure 7, right schematic). Thus, it may well be that the CEM15/APOBEC3G-mediated dC→dU deamination in first-strand cDNA that is read out in the experimental system used here in terms of G→A hypermutation of the plus strand of DNA could function under physiological conditions to limit the spread of viral infection by triggering a uracil-based excision pathway.

The concept established here of an innate nucleic acid deamination mechanism may well predate the adaptive program of antibody gene diversification (Neuberger et al., 2003) and could easily apply to other types of viruses. This defense mechanism seems also a likely contributor to viral sequence variation.

Experimental Procedures

Plasmids and Cells

The pHIT60, pczVSV-G, pCFG2fEYFPf, and pCEM15:HA vectors have been described (Bock et al., 2000; Sheehy et al., 2002). pcVIF is a pBC12-based vector containing the HIV-1_{IIIB} *vif* gene as an XbaI-SalI restriction fragment and four downstream copies of the M-PMV CTE. MLV-GFP was the kind gift of F. Randow (Cambridge, UK). A nonepitope-tagged human CEM15/APOBEC3G cDNA (Harris et al., 2002) was subcloned using PCR into pIRES-Zeo (Clontech). 293T cell derivatives stably expressing CEM15/APOBEC3G were selected

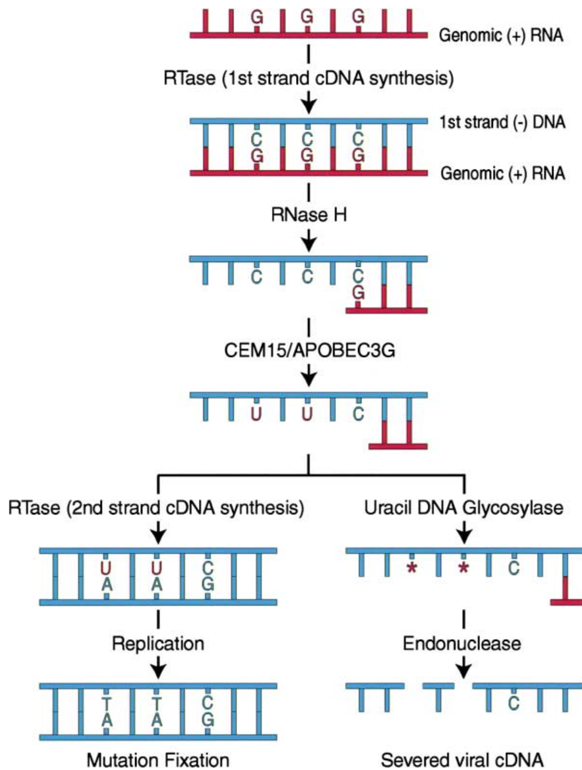


Figure 7. Mechanism of CEM15/APOBEC3G Triggered Innate Immunity

Two possible models are contrasted, one illustrating viral inactivation by excessive mutation (left; dU is recognized as dT by DNA polymerases) and the other envisioning viral destruction being furthered by recognition of uracil in DNA (right; a development of a previous speculation; Harris et al., 2003). Deoxyuridine in the first-strand cDNA would be a target for excision by uracil-DNA glycosylase, generating an abasic site (asterisked), and therefore a probable target for endonucleolytic cleavage. However, the identity of this endonuclease is uncertain but is presumed to be analogous to the apurinic/apyrimidinic endonucleases that act on abasic sites in dsDNA in the base excision repair pathway (Lindahl and Wood, 1999). A third possibility suggested by the results of Klarmann et al. (2003) is that the presence of deoxyuridine in minus (first)-strand cDNA may alter the specificity of initiation of plus (second)-strand cDNA synthesis. RTase is reverse transcriptase.

using 250 μg of zeocin and expression confirmed by Northern blot analysis.

DNA Deamination Assays

Recombinant His_8-tagged CEM15/APOBEC3G was expressed in *E. coli* as previously described (Harris et al., 2002; Petersen-Mahrt and Neuberger, 2003) for APOBEC1 and purified on Ni-ATA–Sepharose (Qiagen). DNA deamination was monitored using the UDG-based assay with biotinylated oligonucleotides SPM167 and SPM168 (Petersen-Mahrt and Neuberger, 2003). Isolated histidine-tagged proteins were subjected to SDS-PAGE, transferred to PVDF membrane, and detected using goat anti-His antibody (Santa Cruz) and chemiluminescence.

Viral Infections

Recombinant MLV stocks were produced in 293T cells (Bock et al., 2000) and quantified according to relative levels of RT in supernatants (Cavidi Tech). Approximately one-sixth confluent monolayers of *Mus dunni* fibroblasts or human 293T cells were challenged with varying doses of YFP- or GFP-encoding MLV stocks, maintained for ~48 hr, and examined by flow cytometry (Becton Dickinson) using mock-infected cells as negative controls (Bock et al., 2000).

Retroviral DNA Sequence Analyses

DNA (Qiagen) was purified from populations of GFP-lo and GFP-hi cells isolated by flow cytometry (Cytomation). Gates were established such that only the dullest (GFP-lo) and brightest (GFP-hi) one percent of all cells was collected. A 98% enrichment of the desired populations was achieved as judged by reanalysis using a different FACS. DNA was subject to high-fidelity PCR (Pfu Turbo DNA polymerase, Stratagene) using oligonucleotides 5′-TAGACGGCATCG CAGCTTGGA and 5′-CTGGTGATATTGTTGAGTCA, gel-purified and cloned using internal HindIII and NotI restriction sites. The resulting 730 bp fragment contained the entire GFP coding sequence (+1 to 721, Figure 3). Sequences bearing an identical set of mutations were counted only once. We cannot exclude the possibility that some clones are dynastically related (causing overcounting of some mutations) although this is unlikely to introduce major skewing since the experiments were performed with a replication-deficient virus and the same distributions of hotspots was identified in independent sets of sequences using virus produced from independent CEM15/APOBEC3G-expressing producer cells.

Virion Protein Precipitations

MLV particles were isolated from culture supernatants by centrifugation at 20,000 × g for 60 min. Pellets were resolved by SDS-PAGE and subjected to immunoblot analysis using a goat anti-MLV Gag antiserum (Quality Biotech Inc.) or an anti-HA monoclonal antibody (Covance), HRP-conjugated secondary antibodies and chemiluminescence.

Acknowledgments

We thank R. Grenfell and W. Turnbull for assistance with flow cytometry; F. Randow and J. Stoye for reagents; and A. Lenton for help with figures. We are also grateful for interactions with R.C.L. Beale, J. Butler, J. Di Noia, D. MacDuff, and F. Randow. This work was in part supported by grants from the Medical Research Council (to M.H.M.), the Leukaemia Research Fund (to R.S.H., I.N.W and M.S.N.), the Arthritis Research Campaign (to S.K.P.-M. and M.S.N.) and the Royal Society (to A.M.S.). M.H.M. is an Elizabeth Glaser Scientist supported by the Elizabeth Glaser Pediatric AIDS Foundation.

Received: May 16, 2003
Revised: May 22, 2003
Accepted: May 22, 2003
Published online: May 28, 2003

References

Bock, M., Bishop, K.N., Towers, G., and Stoye, J.P. (2000). Use of a transient assay for studying the genetic determinants of Fv1 restriction. J. Virol. *74*, 7422–7430.

Bransteitter, R., Pham, P., Scharff, M.D., and Goodman, M.F. (2003). Activation-induced cytidine deaminase deaminates deoxycytidine on single-stranded DNA but requires the action of RNase. Proc. Natl. Acad. Sci. USA *100*, 4102–4107.

Chaudhuri, J., Tian, M., Khuong, C., Chua, K., Pinaud, E., and Alt, F.W. (2003). Transcription-targeted DNA deamination by the AID antibody diversification enzyme. Nature *422*, 726–730.

Fisher, A.G., Ensoli, B., Ivanoff, L., Chamberlain, M., Petteway, S., Ratner, L., Gallo, R.C., and Wong-Staal, F. (1987). The sor gene of HIV-1 is required for efficient virus transmission in vitro. Science *237*, 888–893.

Harris, R.S., Petersen-Mahrt, S.K., and Neuberger, M.S. (2002). RNA editing enzyme APOBEC1 and some of its homologs can act as DNA mutators. Mol. Cell *10*, 1247–1253.

Harris, R.S., Sheehy, A.M., Craig, H.M., Malim, M.H., and Neuberger, M.S. (2003). DNA deamination: not just the trigger for antibody diversification but also a strategy for innate defence against HIV? Nat. Immunol., in press.

Janini, M., Rogers, M., Birx, D.R., and McCutchan, F.E. (2001). Human immunodeficiency virus type 1 DNA sequences genetically damaged by hypermutation are often abundant in patient peripheral blood mononuclear cells and may be generated during near-simultaneous infection and activation of CD4(+) T cells. J. Virol. *75*, 7973–7986.

Jarmuz, A., Chester, A., Bayliss, J., Gisbourne, J., Dunham, I., Scott, J., and Navaratnam, N. (2002). An anthropoid-specific locus of orphan C to U RNA-editing enzymes on chromosome 22. Genomics *79*, 285–296.

Klarmann, G.J., Chen, X., North, T.W., and Preston, B.D. (2003). Incorporation of uracil into minus strand DNA affects the specificity of plus strand synthesis initiation during lentiviral reverse transcription. J. Biol. Chem. *278*, 7902–7909.

Lecossier, D., Bouchonnet, F., Clavel, F., and Hance, A.J. (2003). Hypermutation of HIV-1 in the absence of the Vif protein. Science *300*, 1112.

Lindahl, T., and Wood, R.D. (1999). Quality control by DNA repair. Science *286*, 1897–1905.

Madani, N., and Kabat, D. (1998). An endogenous inhibitor of human immunodeficiency virus in human lymphocytes is overcome by the viral Vif protein. J. Virol. *72*, 10251–10255.

Neuberger, M.S., Harris, R.S., Di Noia, J., and Petersen-Mahrt, S.K. (2003). Immunity through DNA deamination. Trends Biochem. Sci., in press. Published online May 23, 2003. 10.1016/S0968-0004(1003) 00111-00117.

Petersen-Mahrt, S.K., and Neuberger, M.S. (2003). In vitro deamination of cytosine to uracil in single-stranded DNA by APOBEC1. J. Biol. Chem. *278*, 19583–19586.

Sheehy, A.M., Gaddis, N.C., Choi, J.D., and Malim, M.H. (2002). Isolation of a human gene that inhibits HIV-1 infection and is suppressed by the viral Vif protein. Nature *418*, 646–650.

Simon, J.H., Gaddis, N.C., Fouchier, R.A., and Malim, M.H. (1998a). Evidence for a newly discovered cellular anti-HIV-1 phenotype. Nat. Med. *4*, 1397–1400.

Simon, J.H., and Malim, M.H. (1996). The human immunodeficiency virus type 1 Vif protein modulates the postpenetration stability of viral nucleoprotein complexes. J. Virol. *70*, 5297–5305.

Simon, J.H., Miller, D.L., Fouchier, R.A., Soares, M.A., Peden, K.W., and Malim, M.H. (1998b). The regulation of primate immunodeficiency virus infectivity by Vif is cell species restricted: a role for Vif in determining virus host range and cross-species transmission. EMBO J. *17*, 1259–1267.

Sova, P., and Volsky, D.J. (1993). Efficiency of viral DNA synthesis during infection of permissive and nonpermissive cells with vif-negative human immunodeficiency virus type 1. J. Virol. *67*, 6322–6326.

Strebel, K., Daugherty, D., Clouse, K., Cohen, D., Folks, T., and Martin, M.A. (1987). The HIV 'A' (sor) gene product is essential for virus infectivity. Nature *328*, 728–730.

Teng, B., Burant, C.F., and Davidson, N.O. (1993). Molecular cloning of an apolipoprotein B messenger RNA editing protein. Science *260*, 1816–1819.

Vartanian, J.P., Meyerhans, A., Sala, M., and Wain-Hobson, S. (1994). G→A hypermutation of the human immunodeficiency virus type 1 genome: evidence for dCTP pool imbalance during reverse transcription. Proc. Natl. Acad. Sci. USA *91*, 3092–3096.

von Schwedler, U., Song, J., Aiken, C., and Trono, D. (1993). Vif is crucial for human immunodeficiency virus type 1 proviral DNA synthesis in infected cells. J. Virol. *67*, 4945–4955.