A Single Amino Acid Determinant Governs the Species-specific Sensitivity of APOBEC3G to Vif Action*

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APOBEC3G (also known as CEM15) is an intracellular antiretroviral factor that is counteracted by the Vif protein of lentiviruses. While APOBEC3G orthologues from several species are active against a broad range of retroviruses, given Vif proteins have a narrow spectrum of activity. For instance, HIV-1 Vif efficiently blocks APOBEC3G from human but not African green monkey (AGM), whereas the reverse is observed with SIVAGM Vif. Here, we demonstrate that a single amino acid at position 128 of human and AGM APOBEC3G governs the virus-specific sensitivity of these proteins to Vif-mediated inhibition. Furthermore, we show that this phenotype correlates with the ability of Vif to bind APOBEC3G and interfere with its incorporation into virions. These results shed light on an important determinant of the tropism of primate lentiviruses.

The replication of a virus within its host and its spread to a new species require that innate lines of defense be overcome. APOBEC3G1 is a cytidine deaminase that confers broad protection against retroviruses and limits the cross-species transmission of these pathogens (1–5). Packaged during viral assembly, APOBEC3G associates with the retroviral reverse transcription complex, where it deaminates cytosine residues in uracil-containing minus-sense DNA (4–7). These U-rich transcripts are either degraded or yield proviruses that are largely non-functional due to G-to-A hypermutation. APOBEC3G is counteracted by the Vif (virion infectivity factor) protein of lentiviruses, which associates with the enzyme to prevent its virion incorporation and trigger its proteasomal degradation (5, 8–12). In the absence of Vif, human APOBEC3G is active against a broad spectrum of retroelements, as it can inhibit the replication of lentiviruses such as human and simian immunodeficiency virus (HIV and SIV, respectively) and equine infectious anemia virus (EIAV), of the gammaretrovirus murine leukemia virus (MLV) (4, 7), and of the hepadnavirus hepatitis B virus (13). APOBEC3G orthologues are also effective against several of these viruses. Vif-defective HIV-1, for instance, is blocked by APOBEC3G from human, rhesus macaque, African green monkey, and mouse (5).

In contrast, a far greater degree of specificity is noted in the Vif sensitivity of these antiviral factors. As an example, the Vif protein of HIV-1 can only counter human and chimpanzee APOBEC3G, but is ineffective against the rhesus macaque, AGM, and mouse orthologues of the enzyme. Conversely, Vif from SIVAGM is active against AGM but not human APOBEC3G (5). Here, we took advantage of these species-specific differences to explore further the mechanism of Vif action.

EXPERIMENTAL PROCEDURES

Expression Vectors—Wild type and vif-defective HIV-1 proviral clones were described previously (14). To permit its trans-expression, a His-tagged form of HIV-1 Vif was inserted into the pEF1/Myc-His plasmid (Invitrogen), yielding the pEF1-VifHis plasmid. The plasmid expressing the HA-tagged form of huAPOBEC3G (3) and the SIVAGM.Vif (pgVif/SIVAGM.Vif) (2) were kind gifts from M. Malim (King’s College, London, UK). The AGM version of APOBEC3G (5) (a kind gift of N. Landau, The Salk Institute, La Jolla, CA) was inserted into the same expression vector as its human counterpart. Chimeras and individual point mutants were constructed using standard subcloning procedures with the help of the QuickChange Mutagenesis kit (Stratagene) when required.

Viral Production and Infectivity Assay—HIV-1 particles were produced by transient transfection of 293T cells with FuGENE (Roche Applied Science) (see www.tronolab.unige.ch for details). For these experiments, APOBEC3G and Vif plasmids were used at a 5- and 2-fold ratio, respectively, compared with proviral construct. Viron release was scored by monitoring the Reverse Transcriptase enzymatic activity in the producer cells supernatant (15). In single-round infectivity assays, viral titer was determined by applying filtered supernatant from producer cells on HeLa-CD4-LTRLacZ indicator cells (16). Virion infectivity was derived by dividing the infectious titer by the amount of physical particles.

Immunofluorescence—After the co-transfection of relevant APOBEC3G-HA plasmid with or without equimolar amounts of HIV-1 Vif-expression vector, 293T cells were cultured on poly-L-lysine (Sigma)-coated coverslips. Cells were fixed with parafomaldehyde, permeabilized with 0.1% Triton X-100, and blocked with PBS, 1% bovine serum albumin. APOBEC3G-HA-specific indirect immunofluorescence was performed as described previously (17), using the monoclonal HA.11 antibody (Covance Research Products, Denver, PA). Low magnification images were taken with a LSM510 confocal microscope (Zeiss). The percentage of APOBEC3G-positive cells was determined by monitoring cells with a fluorescent intensity higher than a threshold set with Photoshop 8 (Adobe). For each condition, two independent transfections were performed, and five whole fields were counted per transfection.

Protein Analysis, Immunoprecipitations, and Virion Purification—The virus producer cells were lysed with radiolabeled precipitation assay buffer. Lysates were precleared (13,000 rpm tabletop spin) and subjected to standard SDS-PAGE. For HA-specific Western blot analysis, the mouse monoclonal 3F10-peroxidase-conjugated antibody (Roche Applied Science) was used. HIV-1 Vif and PCNA were detected with the rabbit anti-Vif 2221 antibody (obtained through the AIDS Research and Reference Reagent Program, Division of AIDS, NIAID, National Institutes of Health, from D. Gabuzda) and monoclonal anti-PCNA antibody (Onogene Research Products, Boston, MA), recognized by the APOBEC3G-HA immunoprecipitated overnight from aliquots of the precleared lysates, using anti-HA affinity matrix (clone 3F10, Roche Applied Science) in PBS. The resulting immuno-
precipitates were washed twice with PBS, once with PBS, 0.025% Triton X-100, once with PBS, 0.25% Triton X-100, and finally once with PBS, 0.25% Triton X-100, 500 mM NaCl final. They were then

**FIG. 1.** Sequence comparison of human and AGM APOBEC3G. The amino acid sequence of the two proteins is depicted in single-letter code. Identical residues are highlighted. An asterisk labels residue 128.

**FIG. 2.** Functional testing of APOBEC3G (APO3G) chimeras and point mutants. A, chimeras depicted on left were tested in a single-round infectivity assay for their ability to interfere with the infectivity of vif-defective HIV-1 and for their sensitivity to inhibition by HIV1-Vif. Results are representative of at least two experiments. B, indicated wild type and point-mutated APOBEC3G molecules (x axis) were tested for their activity against vif-defective HIV-1 (ΔVif HIV-1) and for their sensitivity to HIV-1 or SIV AGM Vif proteins. Infectivity of virions produced in the absence of Vif and APOBEC3G was given the arbitrary score of 100%. Results are representative of at least two experiments.

**FIG. 3.** Morphological and biochemical analyses. A, 293T cells were transfected with vectors expressing HA-tagged forms of wild type (wt) huAPOBEC3G (huAPO3G) or its D128K mutant, together with control or HIV-1 Vif-expressing vectors. The percentage of APOBEC3G-expressing cells was determined by indirect immunofluorescence with HA-specific antibody, as described under “Experimental Procedures.” B, 293T cells were co-transfected with plasmids expressing the indicated HA-tagged APOBEC3G (APO3G) proteins, together with vif-defective or wild type HIV-1 proviral DNA. Cytoplasmic extracts were analyzed by Western blot analysis with HA- and Vif-specific antibodies, without (top panels, the endogenous PCNA protein serving as a control) or with (central panels) prior immunoprecipitation (IP) with HA-specific antibody. The apparently modest effect of Vif on the cellular levels of APOBEC3G (top panel) is due to the significant fraction of cells expressing the enzyme but not the viral protein. Bottom panels, levels of virion-associated APOBEC3G were also assessed by Western blotting, using p24 capsid as a control. No APOBEC3G was detected in the supernatant of cells transfected with APOBEC3G-HA in the absence of the viral plasmid (lane –). The number below each lane indicates the relative infectivity of the virions, giving the arbitrary score of 100% to vif-defective virions produced in the absence of APOBEC3G. Results are representative of two experiments. *hu, human.*
resuspended in Laemmli sample buffer, followed by standard Western blot analysis.

Virions were isolated by ultracentrifugation (200,000 × g, 45 min) through a PBS, 20% sucrose cushion and resuspended in a small volume of TNE (10 mM Tris, pH 8, 0.1 mM NaCl, 1 mM EDTA, pH 8, 1% Triton X-100). Amounts of these lysed virions normalized for reverse transcriptase activity were subjected to standard SDS-PAGE. The same purification procedure was applied to supernatant of cells transfected with APOBEC3G-HA in the absence of a viral plasmid, excluding contamination by nonspecific microvesicles.

RESULTS AND DISCUSSION

Despite their contrasting sensitivities to HIV-1 and SIV<sub>AGM</sub> Vif, human and AGM APOBEC3G present a high degree of sequence homology (Fig. 1). This facilitated the generation of chimeras between these two molecules (Fig. 2A, left). Using a single-round assay, these chimeric derivatives were tested for their ability to inhibit the infectivity of <i>vif</i>-defective HIV-1 and for their susceptibility to HIV-1 Vif-mediated blockade (Fig. 2A, right). Human APOBEC3G (huAPOBEC3G) decreased the transduction efficiency of an HIV-1-derived lentiviral vector approximately 100-fold, while its AGM orthologue (agmAPOBEC3G) exerted an about 10-fold inhibition. As described previously, HIV-1 Vif prevented the action of huAPOBEC3G but was without effect on its AGM counterpart. However, a region encompassing residues 128–133 of huAPOBEC3G was sufficient to confer HIV-1 Vif sensitivity to agmAPOBEC3G. In this region, the two orthologues differ by only two amino acids, at positions 128 and 133 (Fig. 1). Single point mutations were thus introduced in huAPOBEC3G by substituting the corresponding agmAPOBEC3G residue at these two positions. The mutant altered at position 133 exhibited a wild type phenotype (not illustrated). In contrast, huAPOBEC3G<sub>D128K</sub> exhibited the same Vif-sensitivity pattern as agmAPOBEC3G, as it became resistant to HIV-1 Vif, but was effectively blocked by SIV<sub>AGM</sub> Vif (Fig. 2B). To confirm the crucial role of this particular residue, we engineered the reverse mutant, in which lysine 128 of agmAPOBEC3G was replaced by an aspartic acid as found in huAPOBEC3G. The resulting protein, agmAPOBEC3G<sub>K128D</sub> acquired the HIV-1 Vif susceptibility of huAPOBEC3G (Fig. 2B).

A series of additional analyses provided the molecular correlates of these functional data. First, in transiently transfected cells, the immunofluorescence-based detection of wild type huAPOBEC3G, but not of its D128K mutant, was decreased if HIV-1 Vif was co-expressed (Fig. 3A), consistent with a differential susceptibility to Vif-mediated degradation. Moreover, HIV-1 Vif could be immunoprecipitated with and decreased the virion incorporation of wild type huAPOBEC3G and agmAPOBEC3G<sub>K128D</sub> but not wild type agmAPOBEC3G or huAPOBEC3G<sub>D128K</sub> (Fig. 3B). Noteworthy, some huAPOBEC3G and agmAPOBEC3G<sub>K128D</sub> were detected in HIV-1 virions even in the presence of Vif. However, in these experiments where the antiviral was overexpressed, some inhibition of the wild type virus was observed, albeit far lower than measured with ΔVif virus. Remarkably, there was a good inverse correlation between virus infectivity and the residual amounts of virion-associated APOBEC3G.

Together, these results demonstrate that a single amino acid difference between human and AGM APOBEC3G explains the virus-specific sensitivity of these proteins to Vif action. This residue thereby constitutes a crucial determinant of HIV and SIV host ranges and a barrier against the cross-species transmission of these viruses. Another important implication of these data is that the interaction between Vif and APOBEC3G must be direct. While our experiments do not formally define the Vif-binding site of APOBEC3G, our results warrant approaches aimed at identifying drugs that block this interaction, hence the ability of HIV to escape APOBEC3G-mediated innate immunity.

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