LETTER TO THE EDITOR

APOBEC 3F AND APOBEC 3G HAVE NO INHIBITION AND HYPERMUTATION EFFECT ON THE HUMAN INFLUENZA A VIRUS

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APOBEC 3F (A3F) and APOBEC 3G (A3G), the cellular cytidine deaminases belonging to the APOBEC family can induce a deamination of cytosine residues to uracil in DNA or RNA (*1*).

Accumulating evidence indicates that APOBECs also have an antiviral activity against a wide variety of viruses and play an important role in the innate immunity against viral infections (*1, 2, 3*).

Additionally, APOBECs induce HIV genome hypermutation through cDNA cytidine deamination and direct RNA editing. Cytosine-to-uracil editing of APOBECs might contribute to the sequence diversification of RNA viruses in such a way that they do not replicate through DNA intermediation (*4*). Influenza A virus (IAV) is a negative strand RNA virus. A steady increase of uracil and adenine along with the decrease in cytosine and guanine content has been detected in the genomes of human IAV, but not in genomes of avian influenza viruses (*5*). In addition, no APOBEC ortholog has been reported in birds (*5*). One speculation is that humans have a native defense against RNA viruses by possessing APOBECs. Nevertheless, with the exception of retroviruses (*1*), no other RNA viruses have been shown to be inhibited by APOBECs. Since the effect of APOBECs on human IAV is unknown, we have examined the inhibitory and hypermutagenic effects of two major APOBECs, A3F and A3G, on human IAV.

A3F and A3G genes were cloned from phorbol myristate acetate-stimulated peripheral blood monocytes by RT-PCR using a universal forward primer 5'-GTCAAGCTTATGAA GCCTCACTTCAGAAAC-3' and reverse primers 5'-CAGGGTACCTCACTCGAGAATCTCCTG-3' (for A3F) and 5'-CAGGGTACCTCAGTTTTCCTGATTCTGGAG-3' (for A3G). The cloned genes in pcFlag vector were transfected into MDCK cells with Lipofectamine 2000 (Invitrogen) to generate A3F- or A3G- expressing MDCK cells. The expression of A3F and A3G was confirmed by Western blotting and by flow cytometry (results not shown).

MDCK cells transfected with A3F-, A3G-expressing vector or an empty vector were infected with human H1N1 IAV A/Shantou/169/06 (169/06) that was isolated and propagated in MDCK cells at a multiplicity of infection (MOI) of 0.001 and 2 to examine the level of viral replication (*6*). The cells supernatants were harvested at 0, 8, 24, 48, and 72 hrs post infection (p.i.) and the amount of infectious virus was determined by plaque assay on MDCK cells as described previously (*6*). We did not detect any significant

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Abbreviations: APOBEC = apolipoprotein B mRNA-editing enzyme catalytic-polypeptide; A3F = APOBEC 3F; A3G = APOBEC 3G; HA = hemagglutinin; IAV = influenza A virus; MOI = multiplicity of infection; p.i. = post infection

Table 1. IAV infection of MDCK cells expressing A3F, A3G, or empty vector

MOI	Cells expressing	IAV titers as log_{10}PFU/ml* at different time p.i.			
		8 hrs	24 hrs	48 hrs	72 hrs
0.001	A3F	< 1.38	6.13 ± 0.09	7.75 ± 0.13	7.73 ± 0.10
	A3G	< 1.38	6.12 ± 0.12	7.63 ± 0.15	7.66 ± 0.10
	Empty vector	< 1.38	6.03 ± 0.14	7.61 ± 0.13	7.47 ± 0.17
2	A3F	1.91 ± 0.13	6.41 ± 0.15	7.33 ± 0.25	6.90 ± 0.10
	A3G	1.92 ± 0.21	6.52 ± 0.10	7.42 ± 0.17	6.92 ± 0.13
	Empty vector	1.88 ± 0.06	6.51 ± 0.06	7.43 ± 0.11	6.90 ± 0.07

*Mean ± SD; n = 3.

difference in viral replication between IAV-infected cells expressing A3F or A3G and the control infected cells at any phase of infection regardless the cells were infected at low or high MOI. To confirm the expression of APOBEC proteins during infection, IAV-infected A3F- or A3G-expressing MDCK cells were harvested at 0, 3, 8, 24, and 48 hrs p.i. and the expressed proteins were analyzed by Western blotting. We found that proteins A3F and A3G were over-expressed during infection. These results indicated no inhibition of IAV replication by A3F or A3G proteins.

To examine the A3F- and A3G-induced viral mutation, MDCK cells transfected with A3F-, A3G-expressing vectors or an empty vector were infected with IAV 169/06 at an MOI of 0.001. The viral RNA was extracted using QIAamp viral RNA mini kit (Qiagen) and cDNA was synthesized by reverse transcription using SuperScript III (Invitrogen) with RT primer (5'-AGCAAAAGCAGG-3'). The hemagglutinin (HA) segment of viral genome was amplified with HA primers (forward primer 5'-TATTCGTCTCAGGGAGCAAA AGCAGGGG-3'' and reverse primer 5'-TCATATTC TGCACTGTAAAGACCCATTGG-3') using Pfu-Ultra DNA polymerase (Stratagene). A-tag was added to the PCR products using dATP and Ex Taq DNA polymerase (Takara) and cloned into pGEM-T easy vector (Promega). Fifteen individual clones were picked randomly and sequenced by ABI 3730 genetics analysis system (Applied Biosystems). Sequencing of the cloned viral HA segments (8,500 bp) recovered from the infected cells revealed only 4 point mutations induced in A3F- or A3G-expressing cells. Conversely, 3 point mutations were detected on HA segments in the empty vector-transfected cells. Thus, no significant difference in mutation rate among the IAV-infected cells expressing or not expressing A3F and A3G indicated that two important members of APOBECs (A3F, A3G) had no hypermutagenic effect on IAV.

Sequence variation of the viral genome can be associated with a viral escape from the host immune responses and resistance against antiviral drugs (*4*). Cytosine-to-uracil editing could be induced by APOBEC proteins and other cytidine deaminases. We found that A3F and A3G could not hypermutate human IAV and whether other APOBECs, cytidine deaminases or RNA editases play a novel endogenous role in the evolution of viruses is an issue worth being explored.

In conclusion, we have shown that A3F and A3G have no effects on the replication or hypermutation of human IAV.

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