Incorporation of Mouse APOBEC3 into Murine Leukemia Virus Virions Decreases the Activity and Fidelity of Reverse Transcriptase

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APOBEC3 proteins are restriction factors that induce G→A hypermutation in retroviruses during replication as a result of cytidine deamination of minus-strand DNA transcripts. However, the mechanism of APOBEC inhibition of murine leukemia viruses (MuLVs) does not appear to be G→A hypermutation and is unclear. In this report, the incorporation of mA3 in virions resulted in a loss in virion reverse transcriptase (RT) activity and RT fidelity that correlated with the loss of virion-specific infectivity.

In order to examine the effects of mA3 incorporated into MuLV virions, we derived clonal cell lines infected with CasFrKP. The 3T3mA3 cells were derived by transfection of a plasmid including the ecotropic MuLV CasFrKP were examined.

Virion-associated mA3 suppresses CasFrKP MuLV infectivity. In order to examine the effects of mA3 incorporated into MuLV virions, we derived clonal cell lines infected with CasFrKP (14). The 3T3mA3 cells were derived by transfection of a plasmid encoding the full-length mA3 derived from the BALB/c mouse strain and was tagged at the C termini with hemagglutinin (HA) (5). Infected clonal cell lines were obtained from 3T3 cells as well as from 3T3 cells expressing mA3 (3T3mA3) and were designated 3T3/CasFrKP and 3T3mA3/CasFrKP, respectively. In agreement with earlier reports (2, 7, 9, 13, 15), the clonal cell line expressing mA3 (3T3mA3/CasFrKP) released virions that had incorporated an easily detectable level of mA3 (Fig. 1).

The infectivity of CasFrKP containing mA3 was compared to that of CasFrKP devoid of mA3 by a focal immunofluorescence assay (FIA) (16) and normalized for virion number using the level of p30 CA protein (Fig. 2A). The specific infectivity of virions released from cells expressing mA3 exhibited over a 90% reduction in infectivity (Fig. 2B), corroborating earlier studies of inhibition by mA3 (2, 3, 6, 8–11).

Decrease in RT activity in virions containing mA3. A recent study examined the efficiency of virion reverse transcription by monitoring the appearance of strong-stop DNA during the course of the RT reaction using virions isolated from C57BL/6 and BALB/c mice as well as those from mA3 knockout (KO) mice (17). Both mouse strains exhibited a similar decrease in RT activity compared to KO mice, indicating an effect of endogenous mA3. C57BL/6 and BALB/c mice express different allelic forms of mA3, suggesting that both forms inhibit RT to a similar extent. These results may reflect a direct effect of mA3 on the enzymatic activity of RT or an indirect effect, such as interference of primer binding.

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from cells not expressing mA3 (3T3/CasFrKP). Error bars represent the standard errors of 3 to 6 determinations. The media of the samples assayed for infectivity and p30. The determinations are expressed as a percentage of the mean specific infectivity exhibited by virions released 8-h interval were determined and normalized to the levels of p30 present in the samples. RT activity was determined for virions contained in 2.5 ml of supernatant (Fig. 3A). Transversion mutations were infrequent; however, in-determined by Student’s unpaired zymatic virions containing mA3 released from 3T3/CasFrKP cells than case with infectivity, the RT activity was significantly lower in exogenous poly(A)/oligo(dT) as the substrate/primer. As was the orimetric assay (Roche Applied Science; no. 11468120910) with exponents of ratios was used to normalize the infectivity and RT activity determinations. (B) The infectivity of virions released from cells during an 8-h interval was determined by the FIA and normalized to the levels of p30 present in the samples. The determinations are expressed as a percentage of the mean specific infectivity exhibited by virions released from cells not expressing mA3 (3T3/CasFrKp). Error bars represent the standard errors of 3 to 6 determinations. The P value determined by Student’s unpaired t test is indicated. P values of <0.05 were considered significant. (C). The RT activities of virions released from cells during an 8-h interval were determined and normalized to the levels of p30 present in the samples. RT activity was determined for virions contained in 2.5 ml of supernatant media of the samples assayed for infectivity and p30. The determinations are expressed as a percentage of the mean specific infectivity exhibited by virions released from cells not expressing mA3 (3T3/CasFrKp). Error bars represent the standard errors of 3 to 6 determinations. The P value determined by Student’s unpaired t test is indicated. P values of <0.05 were considered significant.

FIG 2 Influence of virion-incorporated mA3 on specific infectivity and virion RT activity. (A) Virion p30 was determined from immunoblots of gels of virion proteins contained culture supernatant from 3T3/CasFrKP and 3T3mA3/CasFrKP cells. Ratios of the intensities of the bands determined by ImageJ densitometry are shown below the sets of bands. Two different levels of each sample were loaded, resulting in sets of bands with nearly identical ratios of intensity. The average of the ratios was used to normalize the infectivity and RT activity determinations. (B) The infectivity of virions released from cells during an 8-h interval was determined by the FIA and normalized to the levels of p30 present in the samples. The determinations are expressed as a percentage of the mean specific infectivity exhibited by virions released from cells not expressing mA3 (3T3/CasFrKP). Error bars represent the standard errors of 3 to 6 determinations. The P value determined by Student’s unpaired t test is indicated. P values of <0.05 were considered significant.
of putative mA3 target sequences from the AKV genome to those of the CasFrKP sequence did not reveal substantial differences in the number of preferred sites. It remains unclear why the incidence of G→A mutations was unchanged, while the incidence of other transition mutations was increased in transcripts from the mA3-containing virions.

The results of this study suggest an effect of virion-incorporated mA3 on the RT of the virus that affects the activity of the transcription process as well as the fidelity of the enzyme. A loss of fidelity of RT as a result of mA3 incorporation has not been previously reported and may represent another cytidine deaminase-independent mechanism by which APOBEC proteins act to inhibit retroviral replication. CasFrKP, like other exogenous MuLVs, encodes a glycosylated Gag protein (gGag) that partially counteracts the action(s) of mA3. Stavrou et al. (17) have recently observed an inhibition of RT activity by mA3 that is counteracted by gGag. It would be of interest to determine if the gGag protein influences RT fidelity.

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