Critical reappraisal of the A226V mutation in Chikungunya outbreaks: possible role in increased pathogenesis?

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SUMMARY

CHIKV is a mosquito-transmitted alphavirus responsible for the first autochthonous Italian outbreak in 2007. We previously analyzed 7 CHIKV isolates (5 imported and 2 autochthonous) with respect to the presence of A226V mutation in E1gp. All the isolates showed this mutation except the one imported from India in 2006. Since this mutation has been associated with enhanced replication and fitness in A. albopictus vector, we investigated the possible involvement of A226V mutation in enhanced infection capability in primate cells.

No significant differences were observed between the two isolates either in terms of replication kinetics or in virus yield, on both Vero E6 and C6/36 cells. Moreover, experiment of inhibition of virus replication were performed for both isolates on Vero E6 cells using increasing amounts of recombinant IFN-alpha and virus yield was measured. A dose-dependent inhibition of virus yield for both CHIK isolates was observed, with a different sensitivity to IFN-alpha between the isolate carrying the A226V mutation and the wild type one.

Our results suggest i) that A226V mutation does not influence replication ability in both host species, when using single replication cycle conditions; ii) the differences between wild type and mutated strains may be due to different sensitivity and/or activation ability of innate immune mechanisms.

Chikungunya virus (CHIKV) is a mosquito-transmitted alphavirus belonging to Togaviridae family. Isolated for the first time from a Tanzanian outbreak in 1952, is geographically distributed in Africa, Asia, Indian Ocean Islands, India, (1,2,6,7,9,11,12). CHIKV is also responsible for several imported cases in Southern Europe, giving rise, in 2007, to the first autochthonous European outbreak in Italy (5,10).

Several mutations of E1 glycoprotein are considered as critical for CHIKV pathogenesis, by testing infection capability in primate cells.


to this aim, Vero E6 and C6/36 cells were infected with two CHIKV isolates, one carrying the A226V mutation and one wild type, using single replication cycle conditions. Progeny virus was measured by both quantitative real time RT-PCR and viral infectivity assay.

Figure 1 shows replication kinetics experiments performed on insect cells (A) and primate cells (B) using MOI 10 for both the isolates. Results are expressed as viral RNA (log copies/mL; continous lines) titer and infectivity (log 50% tissue culture infectious dose [TCID50/mL]; dotted lines).

Under single replication cycle conditions virus yield is about 10 times higher in mosquito cells (Figure 1A) as compared to primate cells (Figure 1B).

No significant differences are observed between the two isolates in terms of replication kinetic and virus yield on Vero E6 (Figure 1B) and C6/36 cells (Figure 1A) both using MOI 10 and MOI 0.01 (data not shown).

The time course curve using MOI 10 indicates that replication kinetics peak at 24h post-infection, remaining at plateau level thereafter (Figure 1). The time course curve using multiple replication cycle indicates that replication kinetics peak at 48h post-infection, remaining at plateau level thereafter (data not shown).

A dose-dependent reduction of virus yield in Vero E6 cells by IFN-α, assessed by infectivity and viral RNA titration, is observed for both the isolates (Table 1).

Preliminary results show different sensitivity to IFN-α between the two isolates: the one carrying the A226V mutation seems to be more inhibited from recombinant IFN-α with respect to the wild type.

The presence of A226V mutation seems not to influence the replication kinetics in both host species, both using single and multiple replication cycle conditions.

The difference between the two isolates, one carrying the A226V mutation (A226V) and one with wild type aminoacid (226WT), in terms of virus yield, is not significant either at MOI 10 or at MOI 0.01.

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The differences between wild type and mutated strains may be due to different sensitivity and/or activation ability of innate immune mechanisms. Further characterization is in progress.

Table 1. Inhibition of virus replication by recombinant INF-α

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<th>RNA 226VT</th>
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<th>TCID50 226WT</th>
<th>% reduction</th>
<th>Log reduction</th>
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