Analysis of transcription and translation viral activities of wild type and 5' terminally deleted Enteroviruses in a model of cultured primary human cardiomyocytes

P1752

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Introduction and purpose

- Enteroviruses (EV) including group B coxsackieviruses (CVB), have single-stranded positive RNA, flanked on the 5' end by a non-coding region (5'NC), which is crucial for the initiation and translation of the viral genome. Recently, CVB strains presenting with genomic 5' terminal deletions ranging in size from 15 to 50 nucleotides have been evidenced in heart tissue from patients with idiopathic Dilated Cardiomyopathy (DCM). These deletions could explain how the virus can persist in the heart and lead to the development of DCM (fig.1).
- Our objectives were to perform a comparative analysis of transcription and translation viral activities of wild type (WT) and 5' terminally deleted viral forms in a model of cultured primary human cardiomyocytes.

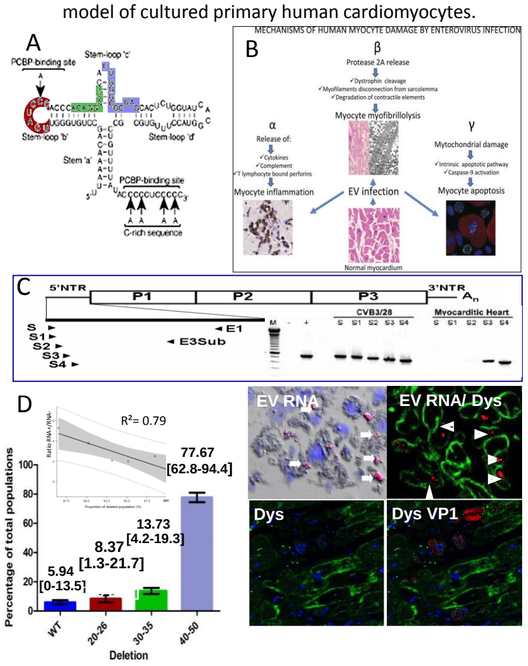

Fig.1 caption :A-5' non coding region. (Sharma, Virology 2009). B-Mechanisms of cardiomyocyte damage after CVB infection (Frustaci, Eur Heart J 2010).C- 5'terminally deleted CVB strains (Chapman Virology 2008).D- left : Wild type (WT) and 5'terminally deleted CVB strains evidenced using next generation sequencing in Endomyocardial Biopsy of a female patient suffering from idiopathic DCM (Bouin EID 2016 submitted); right: EV RNA, Dystrophin (Dys) and Viral protein (VP1) localisation in cardiomyocyte using confocal microscopy (Andreoletti personal data).

Methods

- Δ15 Δ50 and Δ100 viruses (5' terminally deleted of 15, 50 and 100 nucleotides respectively) and 5'untranslated region (ranging from 1 to 601 nt: 5'UTR) were generated by directed mutagenesis from parental WT CVB3 28 reference strain.
- Synthetic RNAs were transfected alone or in association onto (i) human cardiomyocytes (HCM, Promocell GmbH Heidelberg) for viral transcription activity analysis and (ii) Hela cells ATCC (Ref. CCL2) for viral infectious particles quantification using Plaque forming units method.
- Viral RNAs were extracted using NucliSENS® easyMag® (Biomérieux). EV Viral loads were estimated using quantitative Real Time PCR pan-enterovirus. Analysis of translational activities of WT, Δ50 and Δ100 viruses were performed using radiolabeled [S35] Methionin and using CV-B3 replicon expressing emerald green fluorescent protein (emGFP) (Wehbe, J Virol Methods 2016).

Results

- In the supernatant of the cultured human cardiomyocytes from 12 to 48 hours post infection, WT CVB3-RNA levels appeared to be significantly higher than those observed for deleted viral forms (P<0.002) (fig.2A). At 72h p.i., viral RNA loads in the supernatant of cells infected by mix M1 (5% WT and 95% Δ50) were significantly higher than those observed in supernatants of cells infected by Δ50 and Δ100 only and by Mix 2 (5 % WT and 95% non-replicative Δ100 virus) (fig.2B), indicating a potential synergistic effect between the WT and the Δ50 onto viral transcription activities. No infectious particles were detected by plaque forming assays for Δ50 and Δ100 viral forms, whereas viral infectious particles were evidenced in WT and Mixes M1 and M2 (fig.2C&D). Despite an higher viral load than mix M2, mix M1 didn't induce the production of more infectious particles.
- Results of viral replication activites (transcription or translation) of WT, Δ50 and Δ 100 viruses were depicted in fig.3. To further analyse the Helper effect of WT viruses onto the transcription and translation activities of Δ50 viral forms, cardimyocytes were transfected by Δ50 in association with WT or 5'untranslated region (1-601). The ability of Δ50 virus to recombine with WT or 5'UTR or to form a complex with 3CD protein were investigated. All these results are depicted in fig. 4.

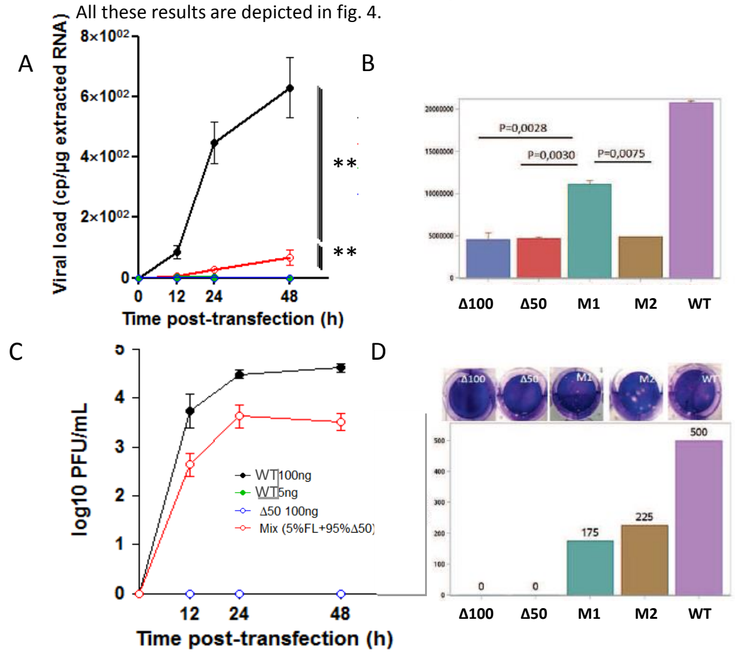

Fig.2 caption: A-CVB viral loads in the supernatant of cardiomyocytes from 12 to 48h after transfection by WT and Δ50 strains alone or in association; ** p<0.05. B-CVB viral loads of WT, Δ50 and Δ100 in the supernatant of cardiomyocytes at 72h p.i. C- viral infectious particles quantification from 12 to 48h after transfection of Hela cells by WT and Δ50 strains alone or in association. D- viral infectious particles quantification of WT, Δ50 and Δ100 at 72h p.i.

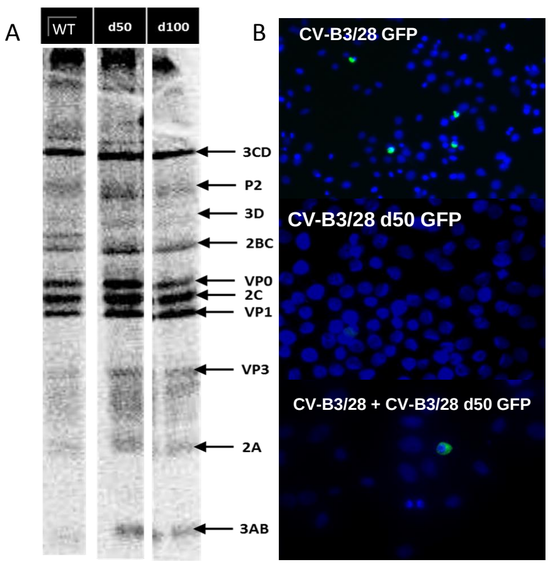

Fig.3 caption: A- In vitro transcribed WT, Δ50 and Δ 100 were incubated with HeLa S10 extract in presence of 35S Methionine. B- Emerald green fluorescent protein (EmGFP) signal (green) in Hela cells transfected by WT and Δ50 CVB3/28 expressing emGFP alone or in association. Cellular nuclei were stained by DAPI (blue).

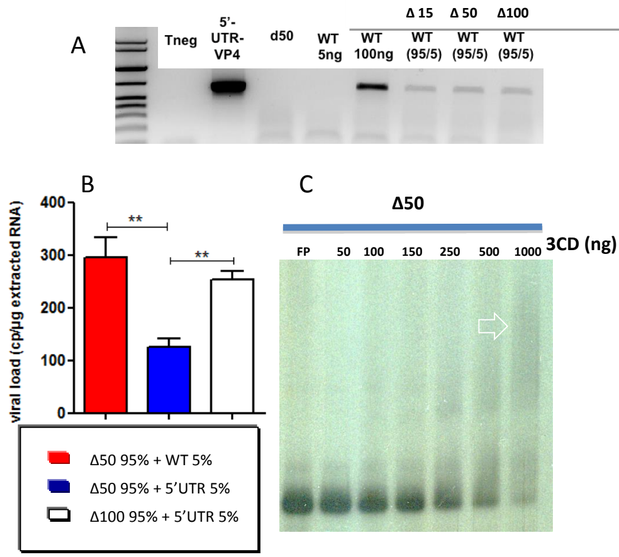

Fig. 4 caption: A- Amplification of CVB RNA between 1 and 601 nt using S1-EntB 5'-ttaaaacagcctgtgggttgttccc-3' (pos : 1-25) and EV1 5'-attgtcaccataagcagcca-3' (pos : 601-581). B- CVB viral loads of Δ50 and Δ100 viruses with Wild type (WT) virus and 5'Untranslated region (1-601nt) (5'UTR) in the supernatant of cardiomyocytes at 48h p.i. **p<0.05. C- Δ50 RNA mobility shift assay with increasing concentrations of 3CD protease-polymerase precursor viral proteins; (N Lévêque personal data). FP Free probe. White arrow showing Δ50 RNA-3CD complex.

Conclusion :

- Our experimental results suggested the existence of a potential synergistic effect ("helper" effect) of the WT virus onto the transcription activity of Δ50 viral forms in cultured human cardiomyocytes.
- This helper effect could play a role in the low viral replication activity involved in EV persistence in human cardiac tissues.

No conflict of interest

Acknowledgments: N Lévêque and B Semler.