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Paper Poster Session
Microbial pathogenesis and virulence

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

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Background: Enteroviruses (EV) 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. These viruses, especially group B coxsackieviruses (CVB), are considered to be a common cause of dilated cardiomyopathy (DCM). Recently, CVB strains presenting with genomic 5' terminal deletions ranging in size from 15 to 50 nucleotides have been evidenced in heart tissue from idiopathic DCM patients. These deletions could explain how the virus can persist in the heart and lead to the development of DCM. 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.

Materials/methods: Δ50 and Δ100 viruses (5' terminally deleted of 50 and 100 nucleotides respectively) 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.

Results: In the supernatant of the cultured human cardiomyocytes from 24 to 72 hours post infection, WT CVB3-RNA levels appeared to be significantly higher than those observed for deleted viral forms ($P<0.002$). 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 Δ50and Δ100 only ($p=0.0030$, and $p=0.0028$, respectively) and by Mix 2 (5 % WT and 95% non-replicative Δ100 virus) ($p<0.0075$), 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 $5.00x10^2$ PFU/ml were evidenced in WT culture. Interestingly, M1 and M2 viral mixes produced similar viral infectious titers estimated respectively to $1.75x10^2$ PFU/ml and $2.25x10^2$ PFU/ml. Despite an higher viral load than mix M2, mix M1 didn't induce the production of more infectious particles.

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.