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siRNA based TLR7/8 activation, MHC class I recycling from endosome and cross presentation of HIV-1 antigen for elevated CD8+ response: An approach for intracellular vaccine

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Background: During chronic HIV-1 infection, INF-1 molecule production decreases upon induction of IFN regulatory factor-1 (IRF-1). On the other hand, HIV-1 Nef mediated Major Histocompability Complex 1 (MHC-1) down-regulation is common in HIV-1 infection hence severely limiting the endogenous antigen presentation. Here we report a synthetic siRNA-fusion peptide complex that uplifts the INF-1 production via TLR7/8 activation and also inhibits MHC class-I (MHC-I) down-regulation, using Tick-borne encephalitis virus soluble Envelope (TBEVsE) as fusogenic peptide, in HIV-1 infected T cells in vitro, thus mitigating the viral replication.

Methods: Co-transfection of 293FT cells with ViraPower™ Packaging Mix (pLP1, pLP2, pLP/VSVG) and pLenti expression vector containing MHC class I. TBEVsE.ShRNA.MS-2- p19MHC-I ($_{LT}$MHC) yielded the lentiviral titer. The control eYFP (enhanced yellow florescence protein) expressing vectors were constructed in similar manner. For the titration of lentiviral stock, the supernatant was harvested from 293FT after 24 hours using 0.22 μm sterile filter. After viral vector construction for $_{LT}$MHC delivery and integration as proDNA in Nef+/Nef- expressing Th cell, pulse-chase assay was done to report the trafficking of HLA-A2 in presence or absence of $_{LT}$MHC in Nef+/Nef-Th cell. Measurement of HLA-A2 transport rate by pulse chase assay was done with endo H digestion. IFN-1 production was evaluated on the basis of ELISA. To quantitate viremia, real-time RT-PCR was performed to measure the number of copies of viral RNA.

Results: Here we report recycling of MHC-I molecule with endogenous HIV-1 antigen attached from late endosome (pH-6.5) with the help of chimeric $_{LT}$MHC with fusion peptide TBEVsE, which facilitated the escape of MHC-I from late endosome thus enabling the molecule to resume the surface expression pathway. TBEVsE complex salvaged heavy chain-β2m heterodimer along with intact HIV-1 antigen. siRNA coupled with p19 protein assisted in activating TLR 7/8 which were confirmed by the increment of INF-β molecules ($P<0.001$) in Nef+/Nef-Th cells.

Conclusions: This study indicates that there are in fact possibilities for intracellular vaccine mechanism which can harness innate as well humoral immune system against infection thus may have potential as a therapy against HIV.

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