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Production of HIV-1 proteins from “defective” HIV-1 provirus

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BACKGROUND

The presence of "defective" proviruses in HIV-infected patients has been well documented (Li et al., 1991; Imai et al., 1991; Sanchez et al., 1997; Ho et al., 2013; Imamichi et al., 2014; Bruner et al., 2016). As these defective proviruses are unable to encode intact viruses, the current consensus view of “defective” proviruses is that these are dead-end products that do not give rise to progeny virus and thus collectively represent “graveyard” of viruses. These “defective” proviruses are thought to be biologically irrelevant and of little significance to HIV-1 pathogenesis. Contrary to this notion, we have recently reported that these "defective" proviruses transcribe novel protein-coding RNA species in HIV-infected patients on combination antiretroviral therapy including those with HIV-1 RNA levels <50 copies/ml (Imamichi et al., 2016). In the present study, we demonstrate the emergence of these defective proviruses during in vitro infection and their association with HIV-1 protein production in the absence of intact virions.

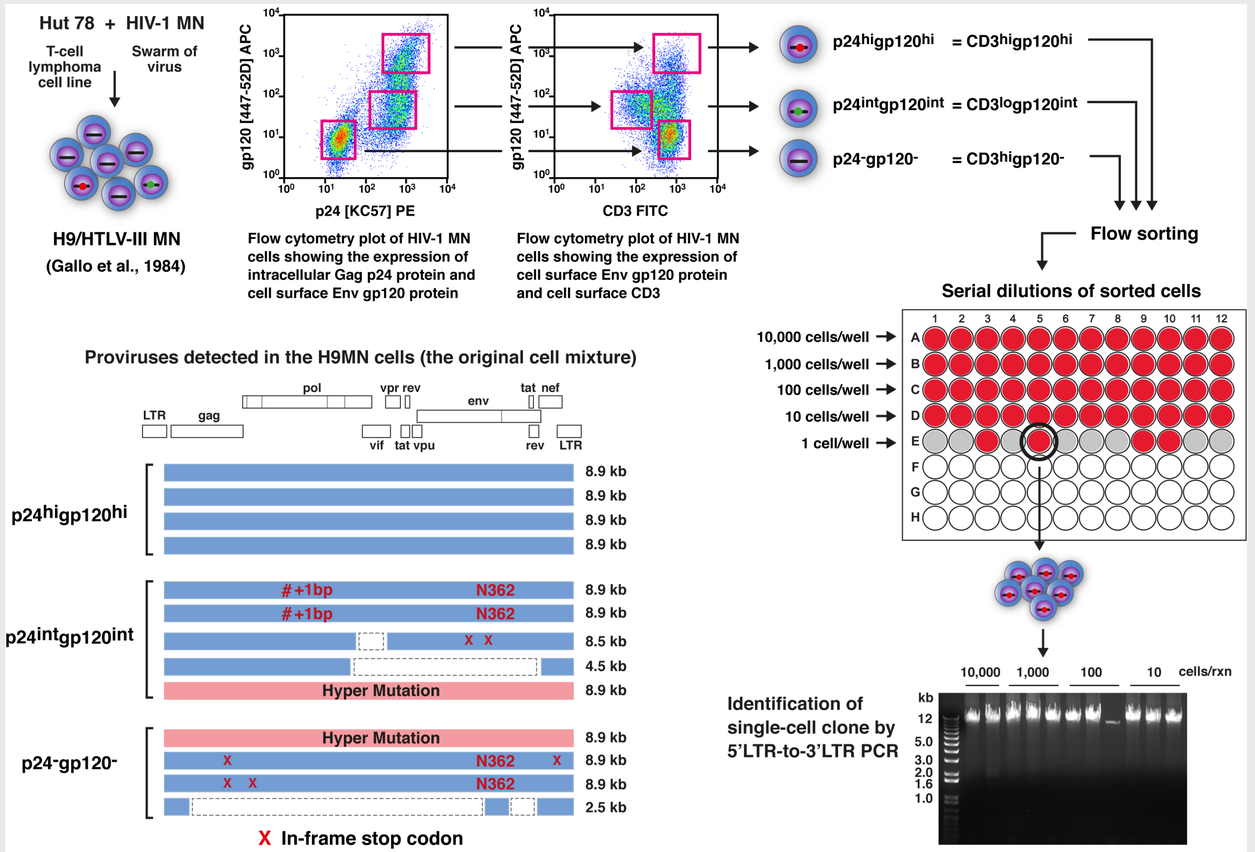
METHODS

Single-cell clones were isolated from an H9 T-lymphoid cell line chronically infected with the MN strain of HIV-1. The H9 culture was first separated based on different cell surface characteristics of CD3 and HIV-1 Env gp120 expression by fluorescence-activated cell sorting. Positive wells by HIV-DNA PCR were further cloned by serial dilution at a cell density of 1 cell per well in 96-well plates. The identification of single-cell clones harboring “defective” proviral DNA was confirmed by combining 5'LTR-to-3'LTR single-genome amplification and direct amplicon sequencing of the genomic DNA as previously described (Imamichi et al., 2016). RNA transcription in the clones of interest was assessed by RT-PCR of near full-length unspliced HIV-1 RNA species. Cellular expression of HIV-1 proteins was analyzed by western blot and flow cytometry.

REFERENCES

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Bruner et al., 2016, Nat Med, 22(9): 1043-1049
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RESULTS

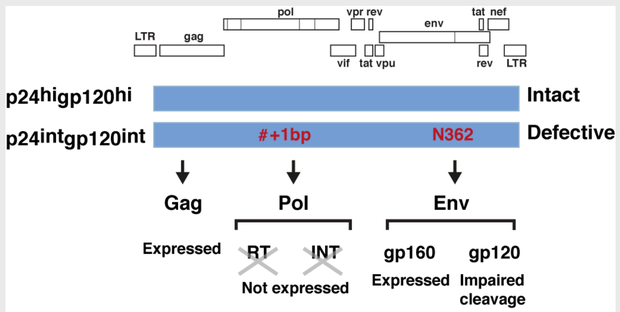


Schematic diagrams of a strategy used to isolate H9MN single-cell clones harboring “defective” proviruses. The H9MN cells were flow-sorted into 3 populations based on cell surface expression of Env gp120 protein and CD3. The sorted cells were serially diluted. The identification of single-cell clones was confirmed by 5’LTR-to-3’LTR single-genome amplification and direct amplicon sequencing. A total of 12 HIV-1 positive individual clones were isolated.

SUMMARY

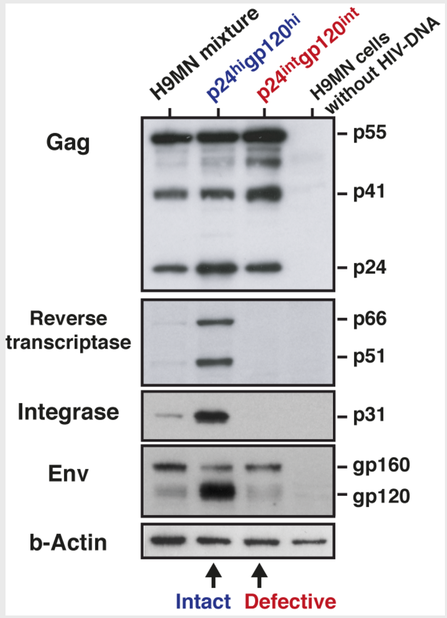
Greater than 95% of proviruses detected in circulating PBMCs of cART-treated patients have been characterized as “defective”, since they lack the ability to encode intact viruses. We have recently shown that these “defective” proviruses are capable of transcribing novel protein-coding HIV-RNA species in patients at all stages of HIV-1 infection (Imamichi et al., 2016). In the present study, we demonstrated the emergence of defective proviruses that closely resembled the “defective” proviral species found in vivo in patients with HIV-1 infection during in vitro infection; and production of HIV-1 proteins from the cells harboring “defective” proviruses. We have referred to these proviruses as "zombie" proviruses (Imamichi, H. et al., International AIDS Conference, 2014), as they are not really "alive" given that they lack the ability to produce intact viruses but can inflict harm by producing foreign nucleic acids and proteins. Persistence of these proviruses may explain the persistent seropositivity to HIV-1 and persistent immune activation seen in patients with "undetectable" virus.

RESULTS



Most prominent among 12 H9MN single-cell clones was the “defective” provirus: p24intgp120int. This “defective” provirus clone had lethal +1 frameshift mutation in the reverse transcriptase (RT) gene (nt 3204 of HXB2), resulting in premature termination of RT translation at Asp 218. Asn 362 (N362) in Env has been associated with decrease in proteolytic processing of the gp160 precursor protein.

RESULTS



Western blots demonstrating cellular expression of HIV-1 proteins in two H9MN single-cell clones: one harboring an intact provirus and the other one harboring “defective” provirus. Consistent with the DNA data, western blots revealed the presence of Gag p55/p24 and Env gp160/gp120 proteins in both clones and a complete absence of RT p66/p51 and integrase protein in the defective provirus clone.

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