HIV-1 Assembly on Intracellular Plasma Membrane-Connected Compartments of Human Macrophages

David O. Nkwe, Annegret Pelchen-Matthews and Mark Marsh

MRC Laboratory for Molecular Cell Biology, University College London, UK.

Introduction

HIV targets key cells in the immune system, such as $CD4^{+ve}$ T-cells and macrophages. In contrast to the cell-surface assembly observed in T lymphocytes and model cell lines, in monocyte-derived macrophages (MDM) HIV-1 assembly and budding appear to occur primarily on intracellular plasma membrane-linked compartments (IPMC) (1-4).

The endosomal sorting complex required for transport (ESCRT) machinery is required for the final scission events that release assembled virus from the plasma membrane in many cell systems. The HIV Gag protein recruits Tsg101 - an ESCRT-I protein (5,7), and ALIX - an ESCRT accessory protein (7,8), to mediate membrane scission during budding.

Aim

To determine whether the IPMC is the only site for HIV assembly in macrophages, we used different strategies to inhibit the ESCRT-mediated scission of HIV in macrophages. This allowed us to generate stable budding intermediates to capture all budding sites in infected macrophages for analysis.

Methods

1. Monocytes were isolated from blood and differentiated to macrophages for 7/8 days after isolation. The cells were either infected on day 7/8.

2. Provirus mutants defective in ESCRT recruitment were generated by site-directed mutagenesis in the Gag p6 subdomain of the wild-type (WT) HIV-1 strain, NL4.3-R3A. Four mutants were made as in Figure 1.

3. Infectious and release defective HIV-1 was produced in 293T cells by rescuing either the $PTAP^{-}$ or $PTAP^{-}YP^{-}$ mutants with Gag WT construct. Purified virus was used to infect MDM. Infected cells were fixed on day 7 post infection and analysed by IF and EM.

Results

1. Mutagenesis of HIV-1 Gag p6

HIV-1 mutants defective in ESCRT recruitment were made and characterised in HEK 293T cells before studies in MDM.

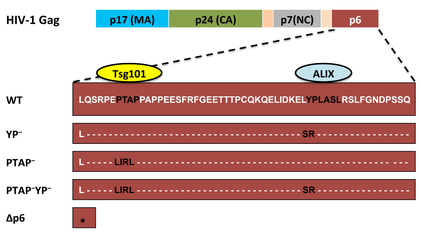


Fig 1. Schematic representation of the Tsg101 and ALIX binding motifs, plus amino acid changes to generate scission defective mutants. These were characterised biochemically and morphologically in HEK 293T cells. The release efficiencies for the $PTAP^{-}$, $PTAP^{-}YP^{-}$ and the Δp6 mutants were 27, 12 and 40%, respectively, suggesting disruption of virus budding. Virus release was modestly reduced to 77% in cells expressing the YP^{-} mutant.

2. Analysis by Immuno-fluorescence (IF)

HIV-1 WT and rescued mutants were used to infect MDM. The cells were then analysed by IF to assess the global distribution of viral proteins.

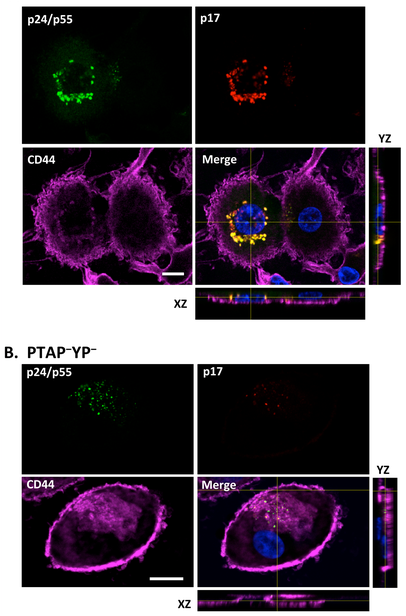


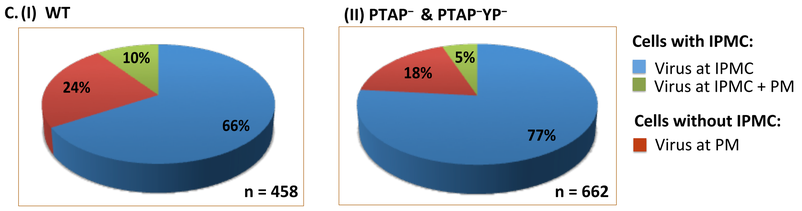
Fig 2. HIV primarily distributes to the IPMC. (A & B) MDM infected with WT or rescued mutant viruses were stained with antibodies to HIV-1 Gag p24/p55 and p17, and the plasma membrane (PM) marker CD44. Anti-p17 is specific for cleaved Gag and therefore stains the mutant very weakly, confirming the budding-arrested phenotype (B).

To quantitate the virus distribution, we scored cells into three categories. Those with virus staining at the:

- IPMC only;
- IPMC and PM (or cell surface); and
- PM only (these cells lacked IPMCs).

A total of 1,120 random cells (458 for WT; 662 for mutants) from eight blood donors were analysed for global distribution of viral proteins (C). Most infected cells had virus staining at the IPMC only, for both the WT (66%) and mutants (77%). A small proportion of cells had virus at both the IPMC and cell surface: 10% and 5% for the WT and mutants, respectively.

Scale bar = 10 μm



For cells with virus at both the IPMC and cell surface, we determined the proportion of Gag p24/p55 staining at these locations. 182 cells from two blood donors were analysed.

A.

Site with fluorescence for virus proteins	Number of cells (%)					
	MΦ D435				MΦ D429	
	R3A WT		R3A PTAP-YP-		R3A PTAP-YP-	
PM (cell surface)	3	(3.4)	1	(2.6)	4	(7.3)
IPMC only	75	(85.2)	35	(89.7)	41	(74.5)
Both IPMC and PM	10	(11.4)	3	(7.7)	10	(18.2)
(a) analysed	6		3		9	
(b) not analysed	4		0		1	
TOTAL	88	(100)	39	(100)	55	(100)

B.

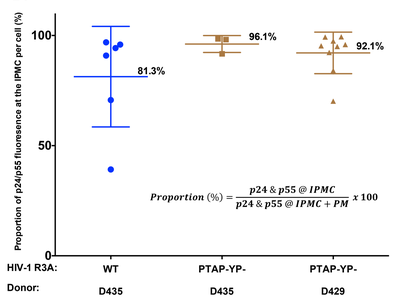
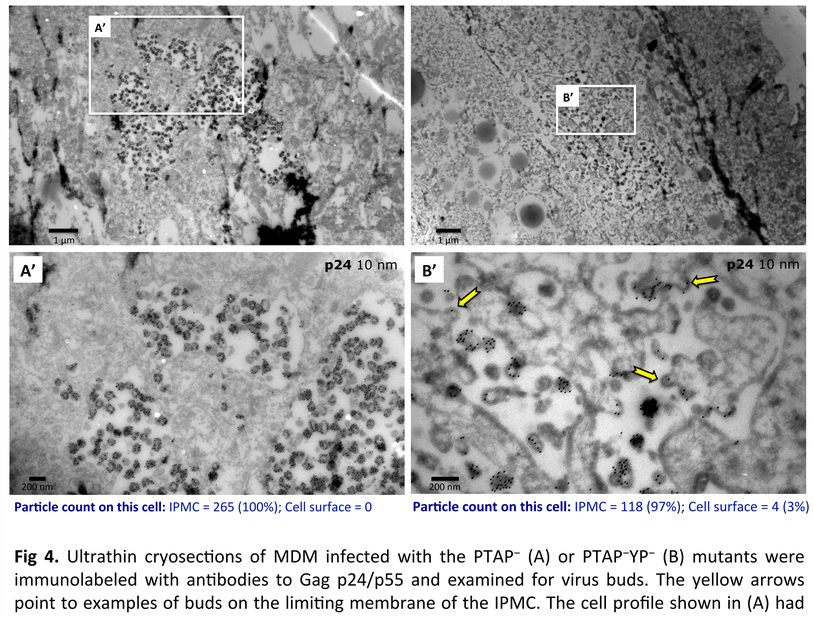


Fig 3. Quantitative analysis of the fluorescence intensity for Gag p24/p55 was done on 3-D images using ImageJ. (A) Cells were scored for virus distribution as above. (B) For those cells where Gag p24/p55 was seen both at the IPMC and PM, ≈90% of the virus fluorescence was at the IPMC.

3. Analysis by Electron Microscopy (EM)

Our IF analysis of MDM indicated that HIV WT and mutants are mainly found at the IPMC. To visualise and quantify budding figures directly, we prepared samples for EM.

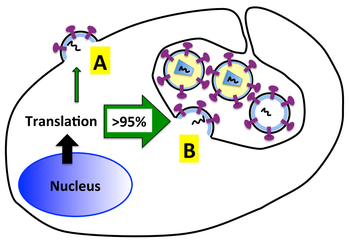


Particle count on this cell: IPMC = 265 (100%); Cell surface = 0

Particle count on this cell: IPMC = 118 (97%); Cell surface = 4 (3%)

Fig 4. Ultrathin cryosections of MDM infected with the $PTAP^{-}$ (A) or $PTAP^{-}YP^{-}$ (B) mutants were immunolabeled with antibodies to Gag p24/p55 and examined for virus buds. The yellow arrows point to examples of buds on the limiting membrane of the IPMC. The cell profile shown in (A) had no immature particles at the cell surface, while for the cell profile in (B), only 4/122 buds were seen at the cell surface. Overall, we analysed 2351 buds on 22 cell profiles and observed 97% of these in the IPMC. Stereology analysis of one cell profile (with 281 buds at IPMC and 3 at PM) showed that there was a similar length of membrane at the surface and lining the IPMC, indicating an enrichment of virus buds in the IPMC of more than 90x.
These EM data confirm our IF observations that HIV-1 preferentially assembles at the IPMC in macrophages.

Conclusion



Model: The IPMC (site B) is the principal site for HIV-1 assembly in human macrophages

This study is the first to provide conclusive evidence that the IPMC is the principal site for HIV-1 assembly in differentiated macrophages (about 2 weeks after isolation). About 80% of cells had virus at the IPMC only. When we visualised individual HIV-1 buds by EM, more than 95% of the viruses were in IPMCs.
As macrophages are long-lived compared to T-cells, this "archiving" of HIV at the IPMC may conceal it from immune surveillance, which may have implications for therapy and disease progression.

References

1. Deneka, M. et al. (2007) J Cell Biol. 23; 177(2): 329–341.
2. Welsch, S. et al. (2007) PLoS Pathog. 3(3): e36
3. Pelchen-Matthews, A. et al. (2012) Traffic 13(2): 273-91
4. Mlcochova, P. et al. (2013) BMC Biology 11: 89
5. Garrus, J.E. et al (2001) Cell 107:55-65.
6. Strack, B. et al. (2003) Cell 114(6):689-99.
7. Weissenhorn W and H. Göttlinger (2011) Cell Host Microbe 9(3):172-4
8. Fisher, R.D. et al. (2007) Cell 128, 841–852

Acknowledgements

Funding:

MRC Medical Research Council

Botswana Government

BIUST BOTSWANA INTERNATIONAL UNIVERSITY OF SCIENCE & TECHNOLOGY