

ORIGINAL

Morphological study on biologically distinct *vpx/vpr* mutants of HIV-2

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Abstract : We have previously shown that human immunodeficiency virus type 2 (HIV-2) without functional *vpx* and *vpr* genes is severely defective for viral growth in lymphocytic cells, and suggested that the virions produced in the absence of Vpx and Vpr are critically damaged. To examine the nature of replication-defect for the *vpx/vpr* double mutant, we quantitatively and morphologically studied the virions produced in cells transfected or infected with wild type clone, single (*vpx* and *vpr* mutants) or the double mutant. While no significant difference in virion production was found for various virus clones in transfected cells, a major growth retardation in infected cells was readily observed for the *vpx* and *vpx/vpr* mutants. In particular, no viral growth was detected for the double mutant. By contrast to the very distinct growth characteristics of the three mutant clones, no appreciable difference in virion morphology was noted. These results indicated that Vpx and Vpr of HIV-2 may cooperatively contribute to virion infectivity without affecting virion morphogenesis. *J. Med. Invest.* 53 : 271-276, August, 2006

Keywords : HIV-2, accessory proteins, Vpx, Vpr

INTRODUCTION

All human and simian immunodeficiency viruses (HIVs and SIVs) isolated so far contain a unique set of accessory genes in their genomes. HIV type 2 (HIV-2) and some of SIVs, such as SIVmac isolated from rhesus monkeys, carry a *vpx* gene in addition to *vpr* (1). Both *vpx* and *vpr* are required for SIVmac to grow optimally in lymphocytic cells (2), and cause AIDS efficiently in monkeys (3, 4). The *vpx* and *vpr* encode small proteins of approximately 100 amino acids which are spe-

cifically incorporated into viral particles (5, 6).

We have previously demonstrated that, in lymphocytic cells, the replication of single *vpx* mutant, but not *vpr*, was impaired, and that of a *vpx-vpr* double mutant was more severely damaged (2). Defective replication sites of the *vpx* single and *vpx-vpr* double mutants were shown to be mapped, respectively, to the nuclear import of viral genome and to both of the nuclear import and virus assembly/release steps. While the mutational effect of *vpr* was rather small, the replication efficiency in one cycle of the *vpx* mutant relative to that of wild-type (wt) virus was estimated to be 10%. Without the *vpx* and *vpr*, the virus replication was negligible. These results have raised a possibility that Vpx and Vpr play an important role(s) for the release and maturation of fully-infectious viral particles. In this report, we have examined the

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level of progeny virion production in transfected and infected cells, and the virion morphology in those cultures by extensive electron microscopic observation.

MATERIALS AND METHODS

Cells

A monolayer cell line 293T (7) was maintained in Eagles's minimal essential medium containing 10% heat-inactivated fetal bovine serum as previously described (8). A lymphocytic cell line HSC-F (9, 10) was maintained in RPMI-1640 medium containing 10% heat-inactivated fetal bovine serum as previously described (8).

Transfection

293T cells were transfected by the calcium-phosphate co-precipitation method as previously reported (8).

Infection

HSC-F cells were infected with cell-free viruses prepared from transfected 293T cells as previously described (11).

Reverse transcriptase (RT) assay

RT assay using ^{32}P -dTTP has been previously described (12).

DNA constructs

An infectious DNA clone of HIV-2 designated pGL-AN has been previously described (2, 13, 14). Proviral mutant clones of pGL-AN designated pGL-St (*vpx* frame-shift mutant, ΔVpx), pGL-Ec (*vpr* frame-shift mutant, ΔVpr), and pGL-St/Ec (*vpx-vpr* double mutant, $\Delta\text{Vpx}/\Delta\text{Vpr}$) have also been previously described (2, 13, 14).

Electron microscopy (EM)

For transfected 293T cells, fixation and embedding were performed according to the method described previously (15, 16). Infected HSC-F cells were centrifuged at 250 \times g and the resultant cell pellets were then washed with phosphate buffered saline before fixation. During the fixation with glutaraldehyde, the pellets were centrifuged again at 1,200 \times g to make the cells more compactly packed. Thereafter, the samples were treated according to the original procedure. The cells embedded in LUVEAK-812 (Nakalai Tesque, Inc., Kyoto, Japan)

were cut into ultrathin sections using a REICHERT-JUNG ULTRACUT E ultramicrotome, doubly stained with uranyl acetate and lead citrate, and examined under a JEOL JEM-1200EX II transmission electron microscope.

RESULTS AND DISCUSSION

Characteristics of virions produced in transfected cells

The human 293T cell line has been frequently and widely used to prepare stocks of various HIV/SIVs because of its high susceptibility to transfection. Furthermore, 293T cells are CD4-negative, and the late phase of virus replication can be easily assessed by monitoring RT production in the culture supernatants. Various proviral clones designated pGL-AN (wt), pGL-St (ΔVpx), pGL-Ec (ΔVpr) and pGL-St/Ec ($\Delta\text{Vpx}/\Delta\text{Vpr}$) were transfected into 293T cells, and virus production was determined on day 2 post-transfection. As shown in Fig.1, all the clones tested here gave similar results upon transfection. Mutations in *vpx*, *vpr* or both did not affect significantly the ability of the virus to release progeny virions as monitored by RT assay. The mutant virions produced in transfected 293T cells were functionally normal since they all exhibited similar infectivity to M8166 cells (data not shown), which are permissive for any *vpx* mutants (2, 13). As shown in Fig. 2, the virions

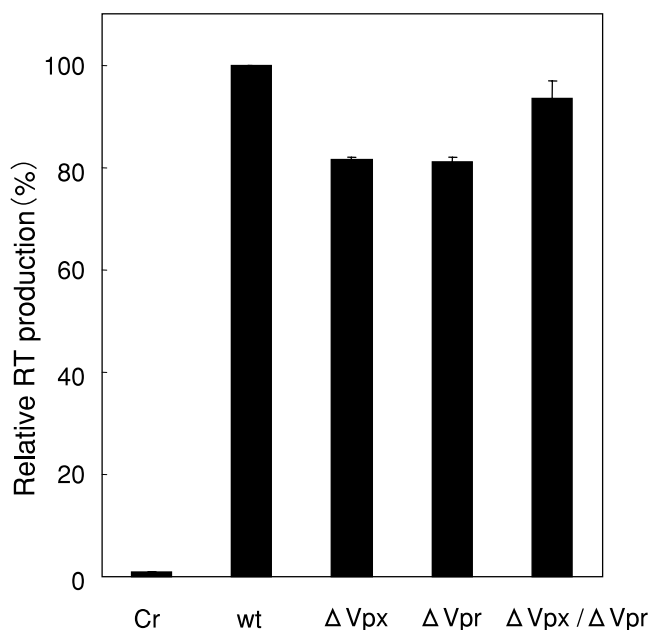


Fig. 1 Progeny virion production in 293T cells transfected with *Vpx/Vpr* mutant clones. Cells were transfected with various proviral clones indicated, and on day 2 post-transfection, virus production was determined by RT assay. RT production relative to that of wt clone is shown.

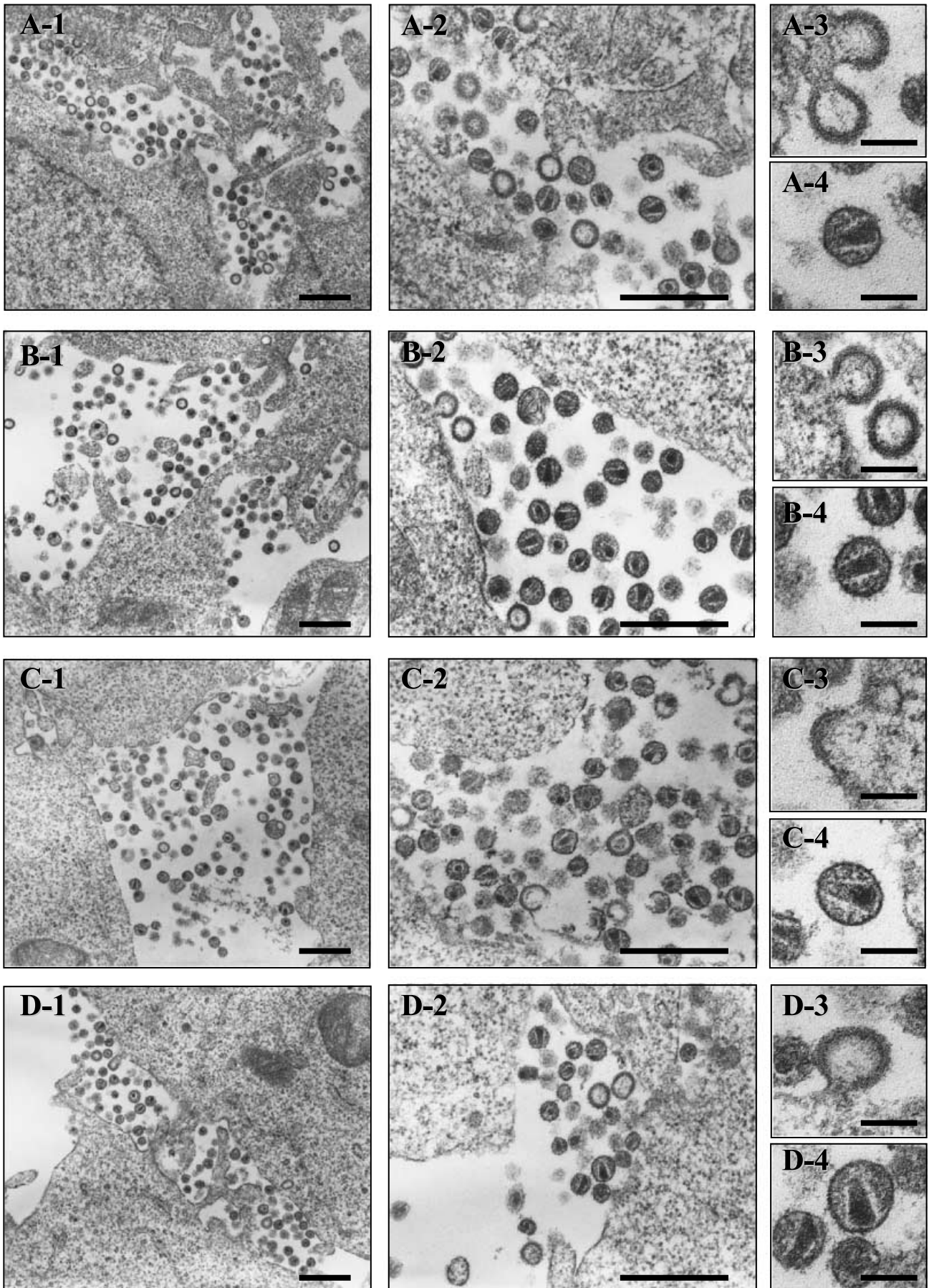


Fig. 2 EM analysis of Vpx/Vpr mutant virions produced in transfected 293T cells. Cells on day 2 post-transfection in Fig.1 were subjected to EM analysis as described in MATERIALS AND METHODS. Samples: A-1 to A-4, wt; B-1 to B-4, Δ Vpx; C-1 to C-4, Δ Vpr; D-1 to D-4, Δ Vpx/ Δ Vpr. Bars : 500nm for A-1 & A-2 to D-1 & D-2 ; 100nm for A-3 & A-4 to D-3 & D-4.

in the cultures were then examined for their morphology by EM. No significant difference of virion morphology was seen among wt and the mutants (A-1 & A-2 to D-1 & D-2). Budding of virions from cells (A-3 to D-3 at a high magnification), immature virions (B-3 at a high magnification), and mature virions with a cone-shaped core (A-4 to D-4) were similarly observed.

Characteristics of virions produced in infected cells

We recently reported that a monkey lymphocytic cell line designated HSC-F behaved exactly like primary human lymphocytes for mutant viruses of HIV-2 (2). We were interested in comparing the morphology of the mutants produced in infected HSC-F cells. Cell-free virus samples were prepared from 293T cells transfected with various clones as above, and an equal amount as determined by RT assay was inoculated into HSC-F cells. As shown in Fig. 3, whereas Δ Vpr grew equally well with wt virus, Δ Vpx displayed a very retarded growth pattern. The double mutant Δ Vpx/ Δ Vpr did not grow significantly during the observation period. The cultures on day 11 post-infection were then subjected to extensive EM analysis. As shown in Fig. 4, the number of virions produced in each culture was significantly different. Cells in-

fectured with wt virus or Δ Vpr generated a large number of virions (A-1 & A-2 and C-1 & C-2) but those with Δ Vpx yielded a relatively small number of progenies (B-1 & B-2). Progeny virions of Δ Vpx/ Δ Vpr were seen only rarely (D-1 & D-2). These observations were in good agreement with the results in Fig. 3. In contrast to the production level of progeny virions, no remarkable difference was noticed for virion morphology among wt and mutant viruses. Immature (A-3 to D-3 at a high magnification) and mature virions (A-4 to D-4 at a high magnification) were seen in all cultures examined.

Conclusion

Our results described here indicated that Vpx and Vpr of HIV-2 do not affect virion morphogenesis appreciably both in permissive (293T) and non-permissive (HSC-F) cells for Δ Vpx. In the absence of Vpx and Vpr, the virus almost lost its infectivity (Fig. 3) but still retained the intact structure as a virion (Fig. 4). We have recently demonstrated, by homology modeling, that HIV-2 Vpx and Vpr are structurally very similar (1). This may account for the cooperative function of Vpx and Vpr. The biological and molecular basis needs to be experimentally determined.

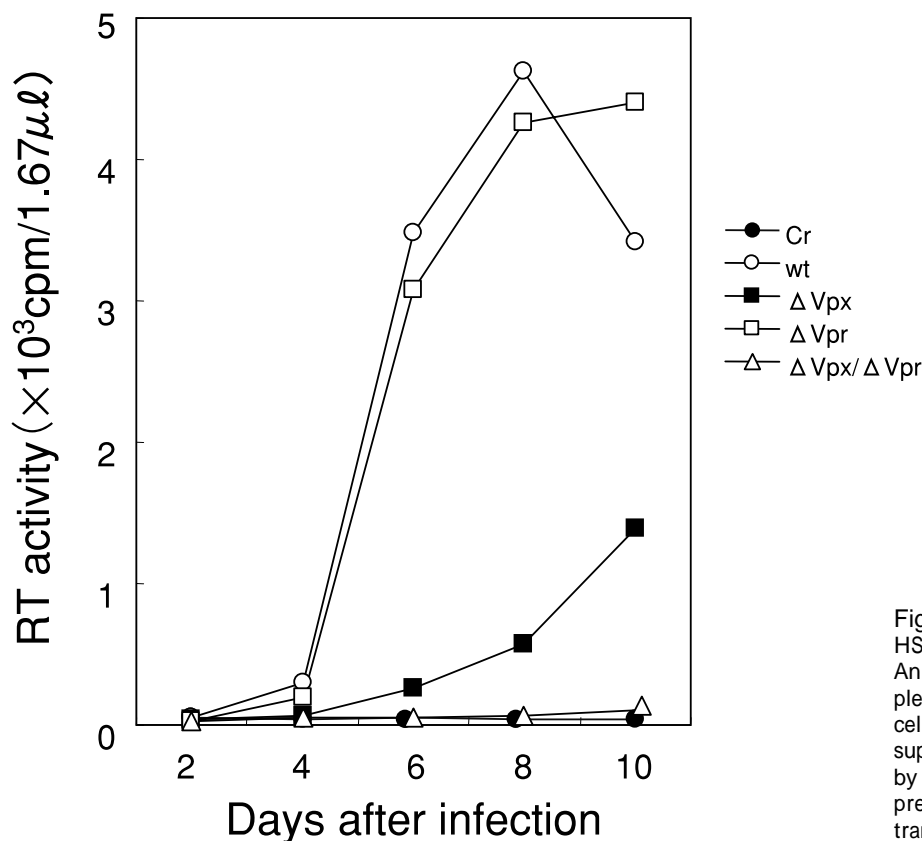


Fig. 3 Growth kinetics in lymphocytic HSC-F cells of Vpx/Vpr mutant viruses. An equal amount of cell-free virus samples indicated was inoculated into HSC-F cells, and virus production in the culture supernatants was monitored at intervals by RT assay. Input cell-free viruses were prepared from 293T cells on day 2 post-transfection.

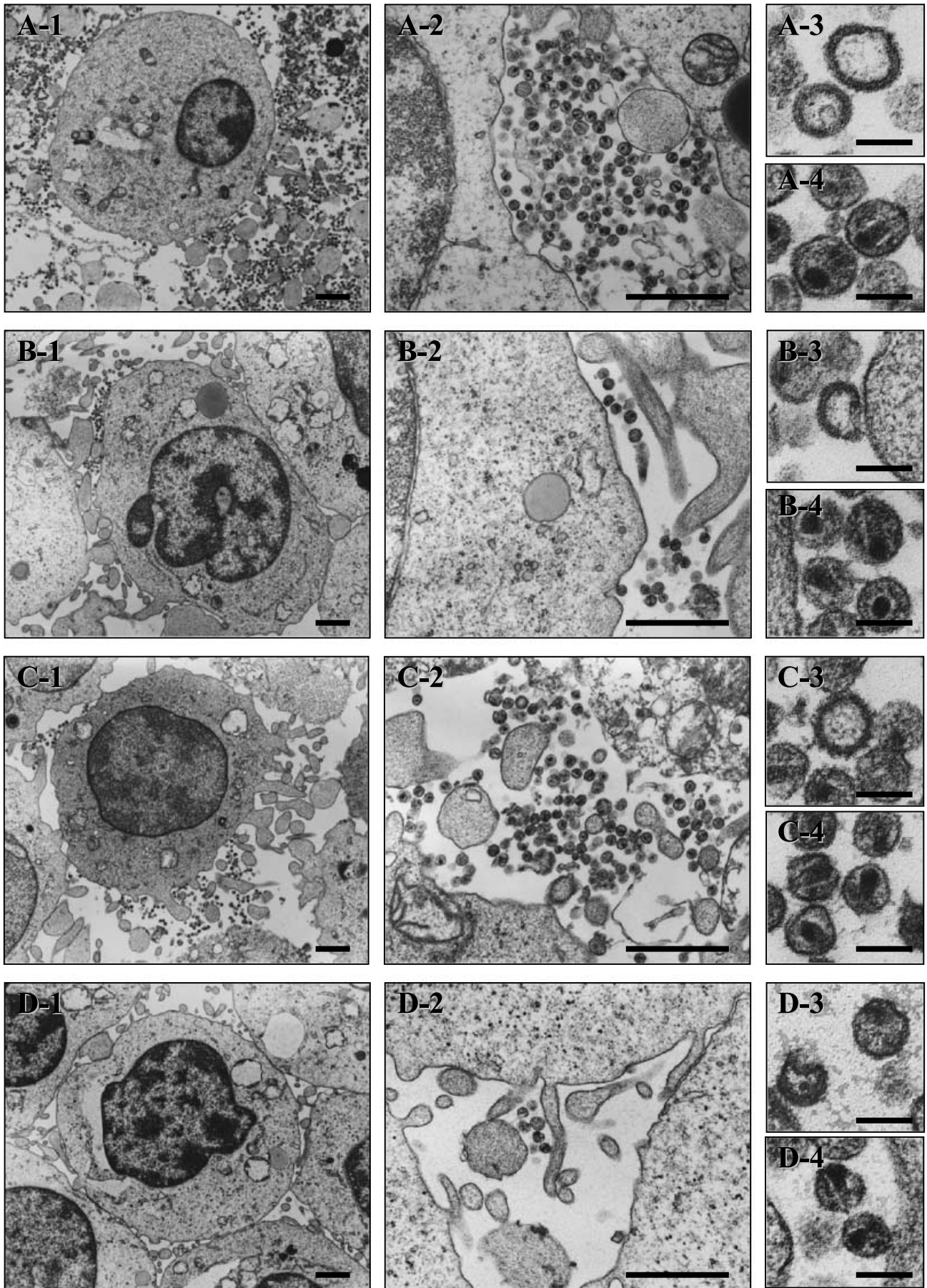


Fig. 4 EM analysis of Vpx/Vpr mutant virions produced in infected HSC-F cells. Cells on day 11 post-infection in Fig.3 were subjected to EM analysis as described in MATERIALS AND METHODS. Samples: A-1 to A-4, wt; B-1 to B-4, Δ Vpx; C-1 to C-4, Δ Vpr; D-1 to D-4, Δ Vpx/ Δ Vpr. Bars: 1 μ m for A-1 & A-2 to D-1 & D-2; 100nm for A-3 & A-4 to D-3 & D-4.

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REFERENCES

1. Khamsri B, Murao F, Yoshida A, Sakurai A, Uchiyama T, Shirai H, Matsuo Yo, Fujita M, Adachi A : Comparative study on the structure and cytopathogenic activity of HIV Vpr/Vpx proteins. *Microbes Infect* 8 : 10-15, 2006
2. Ueno F, Shiota H, Miyaura M, Yoshida A, Sakurai A, Tatsuki J, Koyama AH, Akari H, Adachi A, Fujita M : Vpx and Vpr proteins of HIV-2 up-regulate the viral infectivity by a distinct mechanism in lymphocytic cells. *Microbes Infect* 5 : 387-395, 2003
3. Gibbs JS, Lackner AA, Lang SM, Simon MA, Sehgal PK, Daniel MD, Desrosiers RC : Progression to AIDS in the absence of a gene for *vpr* or *vpx*. *J Virol* 69 : 2378-2383, 1995
4. Desrosiers RC, Lifson JD, Gibbs JS, Czajak SC, Howe AYM, Arthur LO, Johnson RP : Identification of highly attenuated mutants of simian immunodeficiency virus. *J Virol* 72:1431-1437, 1998
5. Selig L, Pages JC, Tanchou V, Preveral S, Berlioz-Torrent C, Liu LX, Erdtmann L, Darlix J, Benarous R, Benichou S : Interaction with the p6 domain of the Gag precursor mediates incorporation into virions of Vpr and Vpx proteins from primate lentiviruses. *J Virol* 73 : 592-600, 1999
6. Accola MA, Bukovsky AA, Jones MS, Gottlinger HG : A conserved dileucine-containing motif in p6^{gag} governs the particle association of Vpx and Vpr of simian immunodeficiency viruses SIV_{mac} and SIV_{agm}. *J Virol* 73 : 9992-9999, 1999
7. Lebkowski JS, Clancy S, Calos MP: Simian virus 40 replication in adenovirus-transformed human cells antagonizes gene expression. *Nature* 12 : 169-171, 1985
8. Adachi A, Gendelman HE, Koenig S, Folks T, Willey R, Rabson A, Martin MA : Production of acquired immunodeficiency syndrome-associated retrovirus in human and nonhuman cells transfected with an infectious molecular clone. *J Virol* 59 : 284-291, 1986
9. Akari H, Nam K-H, Mori K, Otani I, Shibata H, Adachi A, Terao K, Yoshikawa Y : Effects of SIV_{mac} infection on peripheral blood CD4⁺ CD8⁺ T lymphocytes in cynomolgus macaques. *Clin Immunol* 91 : 321-329, 1999
10. Akari H, Fukumori T, Iida S, Adachi A : Induction of apoptosis in *Herpesvirus saimiri*-immortalized T lymphocytes by blocking interaction of CD28 with CD80/CD86. *Biochem Biophys Res Commun* 263 : 352-356, 1999
11. Folks T, Benn S, Rabson A, Theodore T, Hoggan MD, Martin M, Lightfoote M, Sell K : Characterization of a continuous T-cell line susceptible to the cytopathic effects of the acquired immune deficiency syndrome (AIDS)-associated retrovirus. *Proc Natl Acad Sci USA* 82 : 4539-4543, 1985
12. Willey RL, Smith DH, Lasky LA, Theodore TS, Earl PL, Moss B, Capon DJ, Martin MA : *In vitro* mutagenesis identifies a region within the envelope gene of the human immunodeficiency virus that is critical for infectivity. *J Virol* 62 : 139-147, 1988
13. Kawamura M, Sakai H, Adachi A : Human immunodeficiency virus Vpx is required for the early phase of replication in peripheral blood mononuclear cells. *Microbiol Immunol* 38 : 871-878, 1994
14. Kawamura M, Shimano R, Ogasawara T, Inubushi R, Amano K, Akari H, Adachi A : Mapping the genetic determinants of human immunodeficiency virus type 2 for cell tropism and replication efficiency. *Arch Virol* 143:513-521, 1998
15. Uchiyama T, Uchida T : Ultrastructural study on Japanese isolates of spotted fever group rickettsiae. *Microbiol Immunol* 32 : 1163-1166, 1988
16. Sakaguchi T, Uchiyama T, Fujii Y, Kiyotani K, Kato A, Nagai Y, Kawai A, Yoshida T : Double layered membrane vesicles released from mammalian cells infected with Sendai virus expressing the matrix protein of vesicular stomatitis virus. *Virology* 263 : 230-243, 1999