Ultrastructure of Human Immunodeficiency Virus Type 2

By ERSKINE PALMER,* MARY LANE MARTIN,
CYNTHIA GOLDSMITH AND WILLIAM SWITZER

Division of Viral Diseases and AIDS Program, Center for Infectious Diseases,
Centers for Disease Control, 1600 Clifton Road, Atlanta, Georgia 30333, U.S.A.

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SUMMARY

The ultrastructure of human immunodeficiency virus type 2 (HIV-2) was determined by negative stain and thin section electron microscopy (EM). Some virus particles had surface projections about 10 nm in length which were evenly spaced. Nonidet P40-treated particles which were penetrated by stain revealed a distinctive off-centre cone-shaped core and, in addition, free-lying cores were also seen in detergent-treated preparations. The surface of the cores was composed of a layer of small subunits. The structure of HIV-2 determined by thin section EM was the same as that deduced by negative stain EM.

Human immunodeficiency virus type 2 (HIV-2) is a retrovirus which was isolated in 1985 from two carriers living in West Africa who had the acquired immunodeficiency syndrome (AIDS) (Clavel et al., 1986). The virus which causes AIDS primarily in West Africa is being termed HIV-2 to distinguish it from HIV-1, which causes AIDS in countries outside West Africa (Barré-Sinoussi et al., 1983; Gallo et al., 1984).

The morphology of HIV-1 in thin-sectioned cells has been well documented. Mature virus consists of an envelope with fuzzy surface projections surrounding an electron-dense eccentric core that is often bar- or cone-shaped (Munn et al., 1985; Palmer et al., 1985a; Gelderblom et al., 1987). It is similar to retroviruses of the subfamily Lentivirinae, which includes equine infectious anaemia virus (EIAV) and visna virus. The glycoprotein surface projections of HIV-1 are rapidly lost after particles bud from the plasma membrane (Gelderblom et al., 1985, 1987). HIV-1 and HIV-2 have common internal antigens but different envelope glycoprotein antigens. The external glycoprotein of HIV-2 is larger than that of HIV-1. This may account for the more prominent surface projections on HIV-2 when viewed by thin section electron microscopy (EM) (Clavel et al., 1986; Palmer & Goldsmith, 1988). Both viruses have the same type of electron-dense core seen by thin section EM.

To our knowledge, there has been only one report on the morphology of HIV-1 discerned by negative staining EM, the technique most often used to define virus ultrastructure. Stannard et al. (1987) recently described the morphology of HIV-1 stained with phosphotungstic acid. Virions were roughly hexagonal with small ring-shaped surface projections. Virus treated with non-ionic detergent (mucasol) revealed internal components about 90 nm in diameter with some evidence of a central core. HIV-1 could not be differentiated from the oncovirus Friend murine leukaemia virus by negative stain EM. The ultrastructure of HIV-2, however, has not been described in detail. We report here the ultrastructure of HIV-2 as discerned by negative stain and thin section EM.

The ROD2 strain of HIV-2 was obtained from Dr Luc Montagnier, Pasteur Institute, Paris, France. It was propagated in the continuous macrophage cell line U937 (Sundstrom & Nilsson, 1976) obtained from the American Type Culture Collection. Virus was maintained in the cell line by removing one-third of the cell mass and replacing one-third of the used RPMI 1640 medium containing 10% calf serum with fresh medium, every week. Cells were propagated at
37 °C in a 5% CO₂ incubator. Virus was obtained for negative stain EM by centrifuging 10 ml of clarified culture fluid at 39000 r.p.m. for 35 min at 5 °C in a Beckman type 40 rotor. Virus was resuspended in 100 μl cold distilled water and inactivated by adding 100 μl 2.5% glutaraldehyde. This suspension was stored at 4 °C. Portions of the suspension were mixed with 1.0% Nonidet P40 (NP40) and incubated at 4 °C overnight. HIV-2 particles in culture fluid concentrates were prepared for negative stain by the pseudoreplica technique (Smith, 1967) and were stained with 0.5% aqueous uranyl acetate.

For thin section EM, cells were fixed with 2.5% glutaraldehyde in 0.2 M-phosphate buffer pH 7.4, post-fixed in 1.0% osmium tetroxide or in 1.0% osmium tetroxide containing 0.5% ruthenium red, dehydrated through a graded series of alcohols and propylene oxide, and embedded in Epon–Araldite. Sections were post-stained with lead citrate and uranyl acetate. Some samples were treated with 1.0% tannic acid as described by Simionescu & Simionescu (1976).

Most of the HIV-2 particles from culture fluid concentrates that were fixed with glutaraldehyde were generally round and devoid of surface projections. Occasionally we observed evenly spaced surface projections, which measured about 10 nm in length, around the periphery of negatively stained particles (Fig. 1a). Particles averaged about 100 nm in diameter but were sometimes as large as 150 nm. Internal structures were not visible, even though some particles appeared to be partially penetrated by stain. Thin-sectioned particles were also generally round and some had distinct surface projections (Fig. 1b). These projections were enhanced by treatment with tannic acid (Fig. 1c). In addition, staining with ruthenium red emphasized the trilaminar structure of the viral membrane (Fig. 1d).

Glutaraldehyde-fixed culture fluid concentrates mixed with equal volumes of 1.0% NP40 contained particles penetrated by stain, cores devoid of envelopes and partially disintegrated particles. Cores were clearly seen within penetrated particles. Most of these cores were cone-shaped and eccentrically located (Fig. 2a,b,c) and amorphous material filling the remainder of the particle was in evidence. The cone-shaped cores measured about 100 nm in the longest dimension and 45 nm across the base. Cores always lay horizontally and were never seen positioned upright on the base. In thin-sectioned cells, the core of HIV-2 appeared in various forms depending upon the plane of section of the cone. These were always electron-dense and appeared conical when sectioned longitudinally and round when sectioned transversely (Fig. 2d). Preparations of NP40-treated HIV-2 culture fluids also contained free-lying cores. Some of these retained the cone shape, while others appeared to lose this structural integrity (Fig. 3a). The core itself appeared to be enclosed by a layer in which small subunits could be discerned (Fig. 3b). Subunits were seen most clearly around the periphery of the core (Fig. 3c), although a definite structure could not be determined. This layer of subunits is probably analogous to the single membrane around the core of EIAV described by Weiland et al. (1977).

HIV-2 has an ultrastructure which is very similar to that reported for EIAV (Weiland et al., 1977; Gonda et al., 1978). The latter virus has an off-centre cone-shaped core similar to that of HIV-2. The characteristic conical shape of the core makes it easy to distinguish HIV-2 from the oncoviruses that have an electron-lucent central core. However, bar-shaped cores, often seen with EIAV (Weiland et al., 1977), were seldom apparent inside penetrated HIV-2 particles.

HIV-2 was most often seen within cytoplasmic vacuoles in U937 macrophage cells (Fig. 4). In contrast, HIV-1-infected T4 lymphocytes show mostly extracellular particles or particles forming at the plasma membrane (Palmer et al., 1985a,b). Very few particles with surface projections were seen; apparently the projections of HIV-2 are easily lost when virus is manipulated in the laboratory or possibly shed as soon as particles bud from the cell as described for the projections of HIV-1 (Gelderblom et al., 1985, 1987). We and others (I. Chrystie, personal communication) have found that HIV-2 cannot be distinguished from HIV-1 by EM.

Other types of particles seen by negative stain EM were degenerating particles which were smaller round forms of about 80 nm in diameter, with internal amorphous material but no conical nucleoid. These may be intermediate forms of the virus or unidentified viruses of cell culture origin, although no comparable viral structure could be seen in infected cells by thin section EM.
Fig. 1. Negative stain and thin section EM of HIV-2. (a) Virus fixed with glutaraldehyde showing 10 nm surface projections (arrow). (b) Virus in an intracellular vacuole stained with lead citrate and uranyl acetate. Surface projections are clearly visible. (c) Virus was treated with tannic acid before staining, which enhanced visibility of the projections. (d) Virus in an intracellular vacuole stained with ruthenium red, which emphasizes the trilaminar structure of the viral membrane. Bar markers represent 100 nm.

Fig. 2. (a to c) Negatively stained HIV-2 fixed with glutaraldehyde and treated with 1.0% NP40. Particles are penetrated to reveal a cone-shaped core in a horizontal position. (d) Thin section of extracellular HIV-2 showing the electron-dense cone-shaped core in various planes of section. Bar markers represent 100 nm.
Fig. 3. Negative staining of free-lying HIV-2 cores after treatment with NP40. Some have retained the cone shape (arrow in a), while others have lost this cone formation. (b, c) The surface is composed of small subunits that can be more clearly seen around the periphery (arrow in c). Bar markers represent 100 nm.

Fig. 4. Thin section of HIV-2 in cytoplasmic vacuoles of a U937 macrophage cell. Bar marker represents 100 nm.
REFERENCES


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