The Morphology of Human Immunodeficiency Virus Particles by Negative Staining Electron Microscopy

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SUMMARY

Negative staining electron microscopy was used to examine culture fluids from the H9/HTLV-III cell line after concentration by centrifugation. Characteristic retrovirus-like particles bearing distinctive envelope projections were seen. The virion envelope was frequently extended in the form of a bleb or a tail. These particles were morphologically virtually indistinguishable from similar preparations of Friend murine leukaemia virus. H9/HTLV-III culture fluids contained, in addition, numerous comet-shaped particles with a dense head and flared tail. These particles were clumped by the addition of anti-HTLV-III-positive serum suggesting that they may represent intermediate forms of the virus.

The virus implicated as the causal agent of acquired immunodeficiency syndrome (AIDS) has been shown to be a lymphotropic retrovirus and is widely known as lymphoadenopathy-associated virus (LAV) (Barré-Sinoussi et al., 1983) or human T cell lymphotropic virus type III (HTLV-III) (Gallo et al., 1983). Recently (Coffin et al., 1986), a proposal was made by a subcommittee of the International Committee on the Taxonomy of Viruses, that the AIDS retroviruses be officially designated as the human immunodeficiency viruses (HIV).

The morphology and morphogenesis of retrovirus-like particles has been described in ultrastructural studies of thin sections of lymphocytes from AIDS patients or of infected cultures of lymphocyte cells (Palmer et al., 1985a, b; Munn et al., 1985; Lecatsas et al., 1984). These studies have indicated that mature HIV particles consist of an outer envelope, an inner core and a central nucleoid. Although such studies are invaluable for supplying details of sites of virus assembly and release, resolution of surface structural components and an understanding of the three-dimensional shape and size of the virion can best be obtained by electron microscopy of negatively stained particles.

We have used 1% phosphotungstic acid pH 6.2, for negative staining of concentrated preparations of particles obtained from supernatant culture fluids of the H9/HTLV-III cell line supplied by Dr R. C. Gallo (National Cancer Institute, Bethesda, Md., U.S.A.), and maintained in culture by Dr S. F. Lyons (National Institute of Virology, Johannesburg, South Africa). Samples of supernatant culture fluids were concentrated by centrifugation at 38000 g for 60 min in a Spinco SW50.1 rotor. Initial pellets were generally resuspended in 0-02 m-phosphate buffer pH 7.2 (PB) and re-centrifuged to separate the larger virus particles from low molecular weight proteins. Care was taken to avoid unnecessarily high g forces and thus to minimize the disruptive and distorting effects of high speed centrifugation (Polson & Stannard, 1970). Electron microscopy (using a Hitachi-600 electron microscope) revealed a variety of morphological forms, some consistent with those associated with mature retrovirus particles and others which might represent immature or incomplete virions.

Virus-like particles, ranging in diameter from approximately 100 nm to 130 nm, were fairly numerous (Fig. 1a, b, c). These particles were frequently roughly hexagonal in outline and
Fig. 1. (a, b, c) Complete HIV virions of roughly hexagonal shape possess a short fringe of surface projections. Projections can be seen end-on on the top surface of envelope blebs (arrows). (d) Complete FLV virion has a similar size, shape, fringe and membranous envelope protrusion as the HIV particles. Bar marker represents 100 nm for Fig. 1 to 3.

Fig. 2. (a, b, c) Tailed forms of HIV are similar in morphology to tailed FLV particle shown in (d).

Fig. 3. (a, b, c, d) HIV particles after exposure to detergent allow visualization of inner core components and central nucleoid.
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possessed a fringe of short, regular projections. Under favourable conditions of staining, these projections could also be visualized on the surface of particles where they appeared as small ring-shaped units approximately 5 nm in diameter. Membranous 'blebs' or protrusions of the outer coat of the virion were often seen. The surface ultrastructure of protrusions was indistinguishable from that on the rest of the virion. It is possible that some of these protrusions may be caused when the particles are exposed to the high vacuum of the electron microscope and their membranous outer coat partially collapses around a more rigid inner core component. Alternatively, they may represent portions of the cell membrane adjacent to the budding virion which, because they are rich in virus-coded glycoproteins, are budded off together with the virion. This latter hypothetical process might also explain the morphogenesis of the numerous 'tailed' particles seen in the same preparation (Fig. 2a, b, c). The tails had a diameter less than that of the virion head and varied in length up to 500 nm. Membranous blebs on the tail were common (see Fig. 2). Surface projections were seen over the entire particle, both head and tail. The tails are most likely to be formed during the last stage of the budding process, and at that stage would link the mature virion to the cell membrane by a membranous stalk. Demonstration of the tailed forms of virus particles by ultrathin sectioning would rely on fortuitous sectioning along the plane of the length of the tail. To our knowledge this has not been described, but examination of micrographs published by Palmer et al. (1985b) indicates that in a few instances residual tail-like appendages are present on extracellular virus particles, and 'teardrop'-shaped particles described by these authors may represent oblique cross-sections of tailed virions.

The HIV particles, with blebs and tails as described above, were morphologically very similar in appearance to negatively stained preparations of Friend murine leukaemia virus (FLV) (Fig. 1d, 2d) obtained from cultures of Evelyne cells (Moennig et al., 1974) kindly supplied by Dr S. Jooste (University of Pretoria, Pretoria, South Africa). Virus was concentrated and purified on a 20% to 70% (w/v) sucrose gradient and further prepared for electron microscopy in a manner similar to that described for HIV.

The HIV virions were seldom penetrated by the negative stain, but after exposure of the preparation to non-ionic detergent (1% mucosal in PB), internal components approximately 90 nm in diameter could be seen. In some cases there was also the suggestion of a central nucleoid (Fig. 3).

The H9/HTLV-III culture medium also contained large numbers of 'comet-shaped' particles (CSPs) with a dense 'head' and a sac-like, membranous 'flare' (Fig. 4). The head of these particles was roughly spherical, although often distorted, and about 90 nm across. The CSPs possessed indistinct projections on some of their surfaces but these were unlike the regular fringe seen on the mature virions. Detergent treatment caused partial disruption of these particles and allowed visualization of rather amorphous internal material, somewhat resembling an untidy ball of wool, in the 'head' position (Fig. 5). It is not known to what degree the detergent was responsible for disintegration of the inner component. We suggest that the CSPs might represent products of defective or incomplete virus budding.

A serum obtained from a patient with high levels of antibody to HTLV-III, measured by enzyme-linked immunosorbent assay from Organon Teknika, The Netherlands, and confirmed by immunofluorescence on the H9/HTLV-III cell line, was diluted 1:4 and added to the concentrated H9 culture fluid pellet. After incubation overnight at room temperature, electron microscopical examination could not detect obvious antibody attachment to the intact fringed virus particles, but clusters of the slightly smaller comet-shaped particles, attached head to head, were seen (Fig. 4). After detergent treatment, antibody was seen to bind more readily to the partially disrupted CSPs (Fig. 5) and also to the extruded internal material. This reaction supports the concept that these have a viral origin and probably represent one of the stages of virus maturation or defective virus production. Western blot analysis of the serum (using DuPont reagents; Diagnostic Biotechnology, Singapore) showed a high concentration of antibodies to gp41 and p31. Antibodies to p55 and p64 were also present but to a lesser extent, as well as trace amounts of antibody to p24. Antibodies to gp160 and gp120 were absent. These two large glycoproteins have been shown to be encoded by the env gene of the virus, and it has been suggested by Allan et al. (1985) that gp160 represents the envelope gene precursor protein and
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Fig. 4. 'Comet-shaped' particles from H9/HTLV-III culture fluid are linked head to head after the addition of human antibody to HIV. Bar marker represents 100 nm for Fig. 4 and 5.

Fig. 5. Detergent treatment allows stain penetration of the particles seen in Fig. 4 and visualization of amorphous inner components.

gp120 a mature envelope glycoprotein. The absence of detectable antibodies to gp120 in the patient's serum may explain the observed lack of antibody reaction with surface glycoproteins on the mature virions by immune electron microscopy (IEM). The identity of the antigen involved in the IEM clumping of the unfringed comet-shaped particles must remain speculative.
It is, by deduction, not a mature envelope glycoprotein, although gp41, which has been characterized by Veronese et al. (1985) as a transmembrane envelope protein of HIV, cannot be excluded. The gag proteins, p24 and p55, are other candidates.

Retroviruses, as well as most other viruses which mature by budding, possess an outer coat which is essentially membranous in nature. This allows for a fairly wide degree of pleomorphism regarding the shape of the mature virion as well as any internal components which may be membrane-bound. Although pleomorphic variations may be a fairly constant feature for one type of virus, they are doubtless to be found also in other viruses, especially those of the same family. This report has shown that two quite distinctly related retroviruses, HIV and FLV, could not easily be distinguished, and that criteria of morphology by negative staining were valuable only in establishing the family relationship.

The appearance of negatively stained HIV particles is, thus far, not well documented. Our results show that the virions (which are frequently tailed) possess an outer envelope with a characteristic substructure (surface projections) that surrounds a core and central nucleoid. Hexagonal outlines suggest that the inner components probably possess a geometric symmetry. H9 culture fluids contain, in addition, smaller comet-shaped particles which lack any distinctive surface structure, but appear from IEM experiments to contain viral antigens. Other pleomorphisms have been described by LeCatsas et al. (1986) and we suggest the HIVs manifest a multitude of morphological components and forms which directly relate to their stage of maturation.

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REFERENCES


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