NOTES

Morphological Comparison of Machupo with Lymphocytic Choriomeningitis Virus: Basis for a New Taxonomic Group

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Striking morphologic similarities between Machupo, Tacaribe, and lymphocytic choriomeningitis viruses were found by thin-section electron microscopy. It is proposed that these viruses be brought together into a single taxonomic group.

Machupo virus, etiological agent of Bolivian hemorrhagic fever, produces a chronic, asymptomatic infection characterized by immune tolerance and persistent viremia in the natural host, *Calomys callosus* (8). Virus transmission to man results from contact with infected rodents apparently without the aid of an arthropod vector (6). Machupo is a chloroform-sensitive ribonucleic acid virus, pathogenic for infant hamsters and mice, and is inactivated at pH 5 and below (14). It is related serologically to the Tacaribe virus complex by a cross-reactive, soluble complement-fixing antigen; each virus of the complex is distinct by neutralization test (15).

Machupo virus has been propagated in a number of cell lines (7, 15), but peak infectivity titers seldom exceeded 10^6 /ml. In both animals and man, highest concentrations of virus were found in lymphoid tissues (Johnson, Webb, and Justines, *unpublished data*). With hope of an in vitro parallel, two lines of human lymphoblastoid cells, obtained from J. Kasel, National Institutes of Health, were inoculated with a human spleen isolate of Machupo virus which had been passaged twice in suckling hamster brain. The Raji cell line was derived from cells from a Burkitt lymphoma patient (5) and PGLC 33H, from the blood of a patient with infectious mononucleosis (10). Twenty ml of suspension, containing 500,000 lymphocytes/ml was grown in 8-oz (240 ml) prescription bottles in RPMI medium (9). After virus inoculation, the cultures were incubated at 37 C, and medium was changed at 4 days. Infec-

tivity titers were determined in VERO cell panels by a method previously described (15). At 7 days postinoculation, titers of 10^{10} plaque-forming units (PFU)/ml were obtained. The Machupo-infected lymphoblast cells were centrifuged ($630 \times g$ for 5 min), and cell pellets were fixed in glutaraldehyde, postfixed in osmium tetroxide, dehydrated, and embedded in Araldite-Epon. Sections were stained with uranyl acetate and lead citrate.

Machupo virus particles were observed in large numbers at the periphery of the lymphoblastoid cells (Fig. 1-3). Particles were round, oval, or pleomorphic and ranged in size from 60 to 260 nm (Fig. 4; mean diameter 110 nm); the larger particles were the most pleomorphic. Virus particles consisted of a well-defined unit membrane envelope with closely spaced projections or spikes and an unstructured interior containing varying numbers (one to ten) of electron-dense granules (Fig. 2). These granules were approximately 20 nm in diameter and were indistinguishable in size, shape, and density from ribosomes of the host lymphoblastoid cells. Virus particles were formed by progressive budding from marginal membranes (Fig. 2, 3, 5). At sites of budding, host cell membrane appeared denser than normal, more clearly bilamellar and covered with surface projections. The dense granules occurred within particles before pinching off (Fig. 5). Occasionally, virus particles were found within intracytoplasmic vacuoles consequent to budding from vacuolar membranes (Fig. 6). No apparent

FIG. 1. *Machupo* virus particles, in large numbers, accumulating at the periphery of a PGLC 33H human lymphoblastoid cell. A wide range of particle sizes occurred in all cultures. $\times 34,000$.

FIG. 2. *Machupo* virus in 33H cells. Virus particles consist of a unit membrane envelope with surface projections and an unstructured interior containing dense granules. A budding particle is located at arrow. $\times 114,000$.

FIG. 3. *Machupo* virus budding (arrow) from marginal membrane of a 33H cell and accumulating extracellularly. $\times 114,000$.

FIG. 4. Distribution of particle diameters of Machupo virus propagated in 33H and Raji lymphoblastoid cells. Mean diameter of 122 particles measured is 110 nm.

damage to these cells was associated with virus infection; ribosomes were not abnormally aggregated (1). Consonant with immunofluorescent studies by others (10), herpeslike particles were found in infected and control 33H cells; these had no observable effect upon Machupo virus infection. Mycoplasma were not detected in cells by aerobic or anaerobic culture techniques, nor by electron microscopy.

Lymphoid organs from *C. callosus* with Machupo virus infection were harvested at intervals between 10 and 24 days. Small numbers of virus particles were found in extracellular spaces in most specimens of thymus, spleen, and lymph nodes. Focal concentrations of particles were observed less frequently, usually in close association with cells identified as lymphocytes or lymphoblasts. Budding occurred from marginal membranes of such cells and particles accumulated in close proximity (Fig. 7). Virus particles were identical to those observed in cultured cells; size ranged from 60 to 320 nm (mean 126 nm). No particles having this morphology were found in preparations from uninfected cell cultures or rodent tissues.

To expand these observations to another member of the presently recognized antigenic group, Tacaribe virus (4)-infected VERO cells, obtained from Adrian Chappell, National Communicable Disease Center, were sectioned and examined by electron microscopy. Virus particles, similar in size and structure to those found in Machupo-infected cells, were observed budding

from cell membranes and accumulating in intercellular space (Fig. 8). With double staining (uranium and lead salts), characteristic dense internal granules were resolved (Fig. 9).

Similarities in biological and physical properties between Machupo and lymphocytic choriomeningitis (LCM) viruses have been noted previously (8, 13). However, the most striking basis for comparison is the precise morphological resemblance between the viruses. This comparison was made directly by using LCM virus, propagated in cultured mouse peritoneal macrophages (Fig. 10). Virus particles at the periphery of these cells were pleomorphic and bounded by unit membrane; they were covered with surface projections and had dense internal granules (Hirsh, Harrison, and Murphy, unpublished data). Observations were in complete agreement with the comprehensive morphological studies of this virus by Dalton and colleagues (3).

To ensure that the Machupo virus studied was not an LCM contaminant, a portion of the virus pellet was inoculated intracerebrally into LCM-susceptible adult mice. There were no deaths. Further evidence for specificity of the virus inoculum was the neutralization of 10^3 PFU of virus by convalescent, but not acute, phase serum from a human with Machupo virus infection.

Although the Tacaribe group tacitly has been included within the heterogeneous family of "arboviruses," current conversion to precise taxonomic criteria dependent upon physical properties of virus particles demands a reevaluation of this position. The morphological characteristics of Machupo and Tacaribe viruses are distinctly different from those of members of the major arbovirus groups which have been studied (2, 11, 12). Moreover, these viruses do not precisely resemble agents of any other presently recognized group. As concluded by Dalton and his co-workers, LCM virus similarly does not fit into any group, although it appears closest to avian leukosis and murine leukemia viruses in overall design (3). Therefore, we propose that: (i) LCM virus, Machupo, Tacaribe, and immunologically related agents be placed in a single taxonomic group and (ii) the type virus of this group be LCM virus. Organization of these viruses into a family must await evaluation of immunological relationships between LCM and the Tacaribe viruses, and the elucidation of structural details which may be resolved by negative-contrast electron microscopy. Parallel studies on representative viruses serologically related to Machupo virus are in progress.

FIG. 5. Machupo virus budding from a Raji cell. Dense internal granules were incorporated into particles prior to pinching off. $\times 140,000$.

FIG. 6. Machupo virus within intracytoplasmic vacuoles of a Raji cell. Only a small proportion of virus particles were found intracellularly. $\times 71,000$.

FIG. 7. Machupo virus particles accumulating at the periphery of a lymphoblast in the thymus of a *Calomys callosus* rodent. Virus was also found in other lymphoid organs—spleen and lymph nodes. $\times 40,000$.

FIG. 8. Tacaribe virus accumulating intercellularly in VERO cell culture. Budding occurred almost exclusively upon marginal membranes (arrow). $\times 29,000$.

FIG. 9. Higher magnification of Tacaribe-virus infected VERO cell culture in Fig. 3. Tacaribe virus was morphologically indistinguishable from Machupo virus. $\times 114,000$.

FIG. 10. LCM virus in mouse macrophage cell culture. The morphology, size, and mode of development of LCM virus was strikingly similar to that of Machupo and Tacaribe viruses. $\times 82,000$.

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