HTLV-III/LAV PARTICLE-ASSOCIATED PROTEINS: I. VIRUS PURIFICATION, INACTIVATION AND BASIC CHARACTERIZATION

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INTRODUCTION

Human T-lymphotropic virus type III/lymphadenopathy virus (HTLV-III/LAV) is the etiologic agent of acquired immunodeficiency syndrome (AIDS), although cofactors may play a significant role in disease expression. Changes in social behavior may reduce the rate of AIDS transmission, but complete curtailment of the epidemic seems unlikely without immunological or pharmacological intervention. Much effort has been invested in the direct examination of the virus and its infection processes to elucidate promising strategies of intervention, one of which may be vaccination. Detailed information on HTLV-III/LAV components, especially proteins, should aid the rational design of vaccines by focusing attention on those viral constituents likely to induce protective immunity.

In this report, a foundation is laid for the characterization of HTLV-III/LAV particle-associated proteins by describing procedures for bulk purification of the virus and its inactivation without any appreciable modification of the viral polypeptides. By several electrophoretic techniques, these polypeptides are assessed for apparent molecular weights, isoelectric patterns and glycosyl prosthetic groups to yield a particle "fingerprint" of some complexity. Finally, certain virus-associated proteins, for example actin, are tentatively identified based on their biochemical characteristics.

MATERIALS AND METHODS

Virus Purification

HTLV-III/LAV (Electronucleonics Inc., Silver Spring, MD) was obtained by zonal centrifugation of supernatant fluids from HTLV-III infected H-9 cells (H9/HTLV-IIIB) through ribonuclease-free sucrose gradients. Virus harvested from the 1.13-1.18 g/cm² density zone was dialyzed against 0.2M NaCl, 0.01M Tris and 1mM EDTA, pH 7.0 (NTE buffer), diluted to about 2 mg/ml and stored at -70°C prior to secondary purification using equilibrium isopycnic centrifugation through a 20-60% sucrose gradient.
Fractions (1 ml) were taken from the top to the bottom of the gradient and assayed for reverse transcriptase (RT) activity and polypeptide composition as described below.

**Virus Inactivation**

Purified HTLV-III/LAV was inactivated by adding 8 volumes of phosphate-buffered saline, pH 7.2 (PBS), and 1 volume of a solution containing 100 µg/ml of 4'-amino-methyl trioxalen (Calbiochem, La Jolla, CA) and 10% dimethyl sulfoxide (DMSO) in PBS. The mixture containing 200 µg/ml viral protein was exposed in plastic Petri dishes without lids to long-wave ultraviolet light (LWUV; 365 nm) with a minimum radiation flux of 6 x 10³ ergs/sec/cm² at the sample surface. Aliquots of irradiated and untreated mixtures were taken at various times and tested for infectivity by culturing with H-9 cells for 45 days followed by indirect immunofluorescence assessment of acetone-fixed cells using normal or AIDS sera developed with fluorescein conjugated anti-human IgG. Polypeptide integrity of treated and untreated virus was evaluated by polyclaramide gel electrophoresis and ELISA essentially as described except that 4-methyl umbelliferyl phosphate (Sigma Chemical Co., St. Louis, MO) was used as substrate for the alkaline-phosphatase conjugate. The product of conjugate enzymatic activity, methyl umbelliferone, was measured by fluorimetry using a microtiter plate reader (MicroFluor; Dynatech Laboratories, Alexandria, VA).

**Analyses of Viral Proteins**

The polypeptide composition of purified virus particles and the apparent molecular weights of HTLV-III/LAV particle-associated proteins were determined by one-dimensional sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) using discontinuous 12.5% acrylamide slab gels. For certain experiments, samples were not heated or reduced in the presence of SDS-containing lysis buffers. Isoelectric patterns of HTLV-III/LAV polypeptides were analyzed by two-dimensional PAGE using thin-layer, broad-range, polyacrylamide isoelectric focusing slab gels (LKB, Bromma, Sweden) instead of separate tube gels in the first dimension. These slab gels were soaked for 1 hr in a volume of 10M urea containing 2% Nonidet-P40 (NP-40) and 1% 2-mercaptoethanol (2-ME) equal to twice the gel mass. The samples were applied and focused for 1.5 hr at 1400V and 1mA maximum current/gram of gel. The influenza virus, A/USSR/90/77, was coelectrophoresed in some gels to calibrate the focusing dimension.

Western blots were prepared by electroblot transfer of HTLV-III or uninfected H9 cell proteins resolved by SDS-PAGE to 0.45 um cellulose nitrate membranes (Schleicher and Schuell, Keene, NH). After transfer, excess reactive groups on the membranes were blocked with 2.5% highly-purified bovine serum albumin (BSA; #A-7030 Sigma) in PBS containing 0.05% NP-40 (BSA buffer). HTLV-III/LAV particle-associated glycoproteins on Western blots were identified by exposure for 1 hr to Concanavalin-A (20 µg/ml) in PBS/0.05% NP-40 buffer containing 1mM MgCl and 1mM CaCl, washing 5 times for 1 min in this buffer and then exposure to horseradish peroxidase (10 µg/ml) in PBS/NP-40 buffer, again for 1 hr. After 5 more washes, the glycobLOTS were developed using the chromogenic substrate, 4-chloro-1-napthol, which had been dissolved in absolute methanol to a final concentration of 0.3% and then diluted 1:6 with NTE buffer containing 6mM H₂O₂. HTLV-III/LAV particle-associated proteins were tested for reactivity with rabbit antisera by immunoblotting as described using ¹²⁵I-labelled goat anti-rabbit IgG to develop bound immunoglobulin followed by autoradiography.