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15. For virus isolation, samples of freshly drawn, heparinized peripheral blood or bone marrow, yielding a minimum of 10^7 viable cells (greater than 90 percent), are needed. These samples must contain the cells of interest, namely, $OKT4^+$ T cells, which are frequently depleted in AIDS patients.
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Serological Analysis of a Subgroup of Human T-Lymphotropic Retroviruses (HTLV-III) Associated with AIDS

Abstract. *The two main subgroups of the family of human T-lymphotropic retroviruses (HTLV) that have previously been characterized are known as HTLV-I and HTLV-II. Both are associated with certain human leukemias and lymphomas. Cell surface antigens (p61 and p65) encoded by HTLV-I are frequently recognized, at low titers, by antibodies in the serum of patients with acquired immunodeficiency syndrome (AIDS) or with signs or symptoms that precede AIDS (pre-AIDS). This suggests an involvement of HTLV in these disorders. Another subgroup of HTLV, designated HTLV-III, has now been isolated from many patients with AIDS and pre-AIDS. In the studies described in this report, virus-associated antigens in T-cell clones permanently producing HTLV-III were subjected to biochemical and immunological analyses. Antigens of HTLV-III, specifically detected by antibodies in serum from AIDS or pre-AIDS patients and revealed by the Western blot technique, are similar in size to those found in other subgroups of HTLV. They include at least three serologically unrelated antigenic groups, one of which is associated with group-specific antigens (p55 and p24) and another with envelope-related (p65) proteins, while the antigens in the third group are of unknown affiliation. The data show that HTLV-III is clearly distinguishable from HTLV-I and HTLV-II but is also significantly related to both viruses. HTLV-III is thus a true member of the HTLV family.*

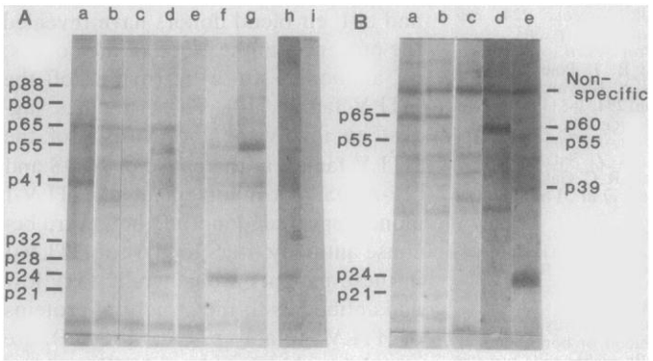
Members of the family of human lymphotropic retroviruses (HTLV) have the following features in common: a pronounced tropism for $OKT4^+$ lymphocytes (*1*), a reverse transcriptase (RT) with a high molecular weight (100,000) and a preference for Mg^{2+} as the divalent cation for optimal enzymatic activity (*2, 3*), and the capacity to inhibit T cell function (*4*) or, in some cases, kill T cells (*5*). Many HTLV also have the capacity to transform infected T cells (*1*). The two major subgroups that have been characterized (*6*) are HTLV-I, which is causatively linked to certain adult T-cell malignancies (*7*), and HTLV-II, which was first identified in a patient with hairy cell leukemia (*8*).

Viruses of the HTLV family have been detected in some patients with the acquired immunodeficiency syndrome (AIDS) (*9*) or with pre-AIDS, a condition frequently progressing to AIDS (*10*). A high proportion of patients with AIDS or pre-AIDS, as well as a significant number of hemophiliacs, have antibodies in their serum that recognize a cell surface glycoprotein (gp61) that is present on certain human T cells infected with HTLV-I (*11*). Gp61 and p65, a slightly larger protein that is a homolog of gp61 and occurs in another cell line producing HTLV-I, were subsequently shown to be related to the HTLV viral glycoprotein (*12, 13*). Studies of blood transfusion recipients who later developed AIDS and of their blood donors have revealed the presence, in the blood of the donors, of antibodies to a retrovirus of the HTLV family (*14*). These findings suggest an involvement of viruses of the HTLV family in the cause of AIDS and pre-AIDS. An involvement of HTLV-I alone appeared doubtful, however, because antibody titers to gp61 of HTLV-I in these patients are generally very low and antibodies to the structural proteins of HTLV, notably p24 and p19 (*15*), are not detectable in most AIDS patients (*16*). Instead, it seemed likely that another member of the HTLV family might be involved in the etiology of AIDS. Here we describe our studies of a group of cytopathic viruses (collectively designated HTLV-III) isolated from patients with AIDS or pre-AIDS. Isolation of these viruses was achieved by means of a novel system permitting the continuous growth of T-cell clones infected with the cytopathic types of HTLV found in these disorders (*17*). We show that antigens associated with human cells infected by HTLV-III are specifically recognized by antibodies in serum from AIDS and pre-AIDS patients, and present a preliminary biochemical and immunological analysis of these antigens.

Lysates of two immortalized and infected human T-cell clones, H4/HTLV-III and H17/HTLV-III (*17*), were tested with samples of human serum in a strip radioimmunoassay (RIA) based on the Western blot technique (*18*). The sera were from patients with AIDS or pre-AIDS, from contacts of such patients, and from homo- or heterosexual male controls. Sera from the same patients were also tested by the enzyme-linked immunosorbent assay (ELISA) with purified HTLV-III as part of a larger, systematic serologic study of the prevalence of antibodies to HTLV-III in AIDS and pre-AIDS patients (*19*).

Representative results are shown in Fig. 1. Sera from patients with AIDS or pre-AIDS, and from some homosexuals and heroin-addicts, recognized a number of specific antigens not detected by sera from heterosexual subjects. The most prominent reactions were with antigens of the following molecular weights: 65,000, 60,000, 55,000, 41,000, and 24,000. Antigens with molecular weights of approximately 88,000, 80,000, 39,000, 32,000, 28,000, and 21,000 gave less prominent reactions. The reaction with the antigen of 55,000 (p55) only occurred in sera that also recognized p24, suggesting a relationship between the two antigens.

The specificity of these reactions was



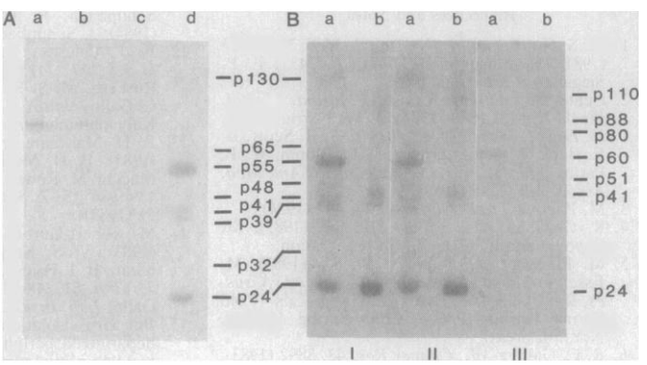


Fig. 1 (left). Serologic detection of antigens in HTLV-III producer cell clones. Strip RIA were performed with human serum as described elsewhere in detail (*21*). Briefly, lysates of HTLV-III producer cell clones were subjected to electrophoresis under reducing conditions on preparative sodium dodecyl sulfate (SDS)–polyacrylamide slab gels, and electroblotted to nitrocellulose sheets (*18*). The sheets were cut into strips. These were incubated with human serum diluted 1:100. After three thorough washings, bound antibodies of immunoglobulin G (IgG) and immunoglobulin M (IgM) classes were made visible with radiolabeled, affinity-purified goat antiserum to human IgG and IgM (H-chain specific) and autoradiography. (A) Analysis with H4/HTLV-III cells. (Lanes a, d, and g) U.S. patients with AIDS; (lane b) a French heterosexual male who developed AIDS after receiving a blood transfusion in Haiti (*24*); (lane c) an AIDS patient from Switzerland; (lane e) a normal heterosexual control; (lane f) a French pre-AIDS patient (*24*); (lane h) a Swiss heterosexual drug addict; (lane i) a normal homosexual control. (B) Analysis with H17/HTLV-III cells. (Lane a) An infant with AIDS whose mother is a prostitute; sera from both are highly positive for antibodies to the HTLV membrane antigen (*11, 25*) and in our ELISA with disrupted HTLV-III (*19*); (lane b) same serum as in (A), lane d; (lane c) normal heterosexual control; (lane d) another Swiss AIDS patient; (lane e) a Swiss heterosexual male intravenous drug abuser with generalized lymphadenopathy and thrombocytopenic purpura (pre-AIDS). Fig. 2 (right). (A) Specificity of the antigens recognized. Lysates of cloned cells before and after infection with HTLV-III were analyzed by the Western blot technique (*18*) with a 1:500 dilution of the serum shown in Fig. 1B, lane e. (Lane a) The H17 clone before and (lane b) the same clone after infection (H17/HTLV-III); (lane c) the H4 clone before and (lane d) the same clone after infection (H4/HTLV-III). All reactive antigens are virus-related with the exception of that with a molecular weight of 80,000 in H17 cells; this antigen binds antibodies from all human sera investigated. Normal human serum did not bind to any of the virus-related bands (not shown). (B) Comparison of antigens in (lanes a) cells and (lanes b) virus. Lysates of H4/HTLV-III (250 μg per lane) or virus purified from the cell culture fluids (*19*) (5 μg per lane) were analyzed with 1:500 dilutions of human sera. (Panel I) Same serum as in Fig. 2A; (panel II) serum of a Swiss male homosexual with fatigue and generalized lymphadenopathy (pre-AIDS); (panel III) serum from same AIDS patient as in Fig. 1B, lane d. An antigen with a molecular weight of 110,000 and p41, p39, and p24 are enriched in the virus preparation [see (*20*)]. The serum in panel III recognized a subset of the antigens recognized by the sera used in panels I and II.

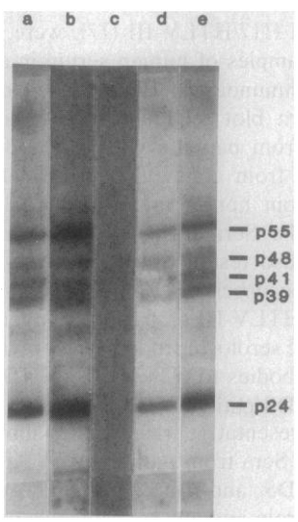


Fig. 3. Relation between HTLV-III and HTLV-II. Serum of an AIDS patient at a dilution of 1:500 was tested in a competition RIA on strips (*20*) prepared with H4/HTLV-III cells. (Lane a) The human serum was added directly to the strip (uncompeted control); (lanes b to e) the serum was first absorbed for 3 hours at 37°C with 1 mg of cellular extract. In (b) the absorption was with uninfected H4 cells (not producing virus); in (c) the absorption was with H4/HTLV-III cells producing HTLV-III (positive control); in (d) the absorption was with C3/44 cells (*26*) producing HTLV-II; in (e) the absorption was with HUT 102 cells producing HTLV-I (*2*).

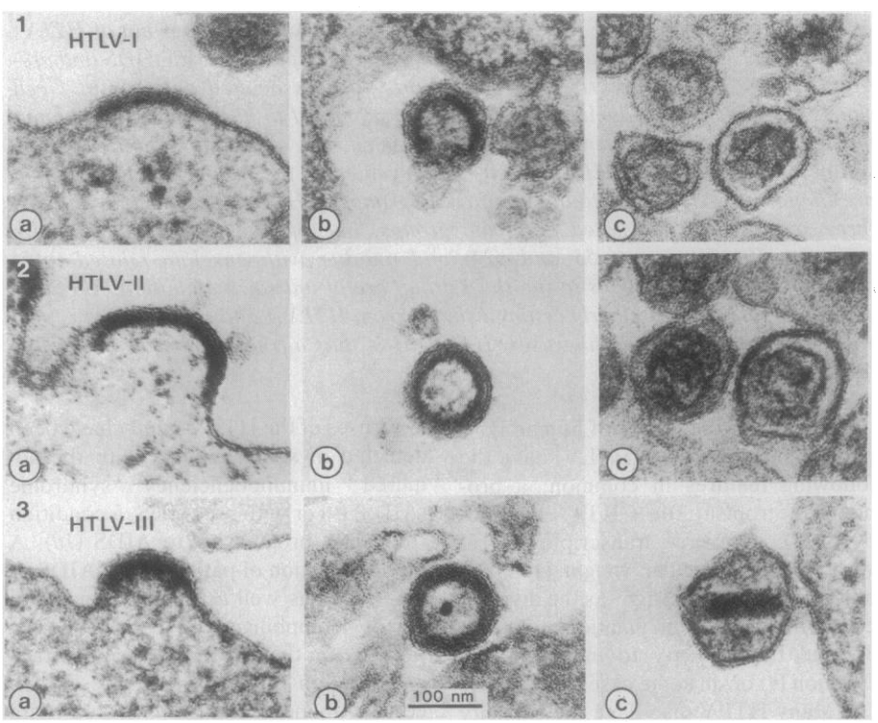


Fig. 4. Electron microscopy of thin sections of cells producing HTLV-I, -II, and -III. (Top) HUT 102 cells producing HTLV-I (*2*). (Middle) Cells from an AIDS patient (J.P.) producing HTLV-II (*24*). (Bottom) Cells from a patient [described in (*27*)] with pre-AIDS, producing HTLV-III. (Panels a) Virus particles budding from the cell membrane. (Panels b) Free particles have separated from the membrane. (Panels c) Free particles sectioned in a different plane. Note the dense, cylindrical core region of HTLV-III.

studied by comparing lysates of H4/HTLV-III and H17/HTLV-III with lysates of the same cell clones, H4 and H17, before viral infection (Fig. 2A). No antigen from the uninfected clones reacted with the sera, with the exception of a protein with a molecular weight of 80,000 in H17 which bound antibodies from all of the human serum samples tested (see Fig. 1B) but not from rabbit or goat serum. Antigens newly expressed after viral infection and recognized by the human serum used for this analysis included p65, p55, p41, p39, p32, and p24. A large protein with a molecular weight of approximately 130,000 and a protein of 48,000 were also detected. With this serum, p55 consistently appeared as a doublet of bands of similar intensity. With normal human serum, none of the antigens was detected (not shown). These results show clearly that the antigens detected after virus infection are either virus-coded proteins or cellular antigens specifically induced by the infection.

The antigens of H4/HTLV-III were also compared with antigens from virus purified from the culture fluids of H4/HTLV-III (Fig. 2B). Extensive accumulation of p24 and p41 [see (*20*)] occurred in the virus preparation (Fig. 2B, panels I and II). Protein stains showed that these molecules are the major components of the virus preparation (*19*). P24 and p41 may therefore be considered viral structural proteins. Furthermore, an antigen with a molecular weight of approximately 110,000 was detected in the virus preparation but was below limit of detection in the cells. Also, p39 [see (*20*)] was present in the virus preparation. It is interesting that p24 in the virus preparation consistently appeared as a doublet (p24/p23), whereas in the cells it appeared as p24 alone. The significance of this is under investigation. P55 was not detected in the virus; however, the intensity of the p55 band in the cells (Fig. 2B, lanes a) appeared to correlate with the intensity of p24/p23 in the virus preparation (Fig. 2B, lanes b), thus again suggesting a relation between these antigens. The p55 is probably a precursor of p24, since a group-specific antigen of similar size (Pr 54^{gag}) in HTLV-I–infected cells is the precursor of p24 and the other *gag*-coded proteins (*21*). Occasionally an additional set of antigens was recognized by a serum (Fig. 2B, panel III) but their relation to the antigens described above is unclear.

Thus we have shown that viral or virus-induced antigens in cloned human T cells infected with HTLV-III are specifically recognized by antibodies in the serum of patients with AIDS or pre-AIDS. The detection of p65 by many of the serum samples is of special interest. We have tested these sera on strips prepared from lysates of cells producing HTLV-I or -II. Some of these cells produce a p65 that has been shown (*13*) to be coded for by the *env* gene of HTLV-I and to be the homolog of the gp61 described by others (*11, 12*). Many of the sera recognizing p65 in HTLV-III–infected cells also recognized, though somewhat faintly, p65 in cells producing HTLV-I or -II, and some of them also recognized *gag*-related antigens (data not shown).

In addition, the reaction of some human sera with virus-related antigens of HTLV-III–infected cells could be partially inhibited by large amounts of extracts of cells producing HTLV-II (Fig. 3). When a human serum not recognizing p65 was used, the antigens for which there was competition included p55, p48, p41, p39, and p24. These results were confirmed by the demonstration that a rabbit antiserum raised against purified HTLV-III showed some reactivity with antigens of HTLV-II and, to a lesser extent, with HTLV-I. In contrast, antiserum to HTLV-II recognized both HTLV-I and -III antigens, and an antiserum to HTLV-I reacted well with HTLV-II, but only faintly with HTLV-III (*22*). Moreover, nucleotide sequences of HTLV-III have been found to be related to HTLV-I and -II (*23*). Although the morphology of HTLV-III particles appears to be somewhat different from the morphology of HTLV-I and -II (Fig. 4), and although some differences are also found in the protein patterns of purified virus preparations (*19*), these immunological and nucleic acid data clearly indicate that HTLV-III is a true member of the HTLV family and that it is more closely related to HTLV-II than to HTLV-I.

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