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7. Dorsal root ganglia from 8-day chick embryos were dissected and trypsinized as described in the legend to Fig. 1, then resuspended at a density of 2.6×10^6 cells per milliliter in phosphate-buffered Gey's balanced salt solution (*p*H 7.4) with bovine serum albumin (1 mg/ml). [^{125}I]NGF (3 ng/ml; specific activity, 11 cpm/pg) prepared by the method of A. Sutter *et al.* [*J. Biol. Chem.* **254**, 5972 (1979)] was added to the cell suspension with or without ethanol (175 mg per 100 ml) and the suspensions were incubated tightly capped for 3 hours at 37°C. Nonspecific binding of [^{125}I]NGF was measured in incubations containing unlabeled NGF (10 μg/ml). Portions of the incubation mixtures were centrifuged for 2 minutes at 10,000*g* in a Beckman Microfuge. After centrifugation the supernatant was removed, the tips of the tubes were cut, and radioactivity was counted in a Searle gamma scintillation counter.
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Characterization of Envelope and Core Structural Gene Products of HTLV-III with Sera from AIDS Patients

Abstract. *The envelope (*env*) and structural (*gag*) gene products of human T-cell leukemia (lymphotropic) virus type III were identified by immunoaffinity chromatography, immunoprecipitation, and two-dimensional oligopeptide mapping methods. The* env *gene specifies a glycosylated polypeptide with a molecular weight of 160,000 (gp160) that is processed to gp120 and smaller gene products. The* gag *gene specifies two polypeptides of 70,000 and 55,000 molecular weight (p70 and p55), both of which contain p24, the major structural protein of the mature virion. The techniques in this study can be used to define the extent of variability of the* env *gene product among different virus isolates and may identify the nature and patterns of the humoral immune response that lead to an immunologically protected state.*

Human T-cell leukemia (lymphotropic) virus type III (HTLV-III) is a human retrovirus closely related in a number of its properties to HTLV-I and HTLV-II (*1–8*). In addition, studies have suggested that this cytopathic virus is more closely related to visna virus, a pathogenic retrovirus in the subfamily Lentivirinae, than to any other retrovirus (*9*). HTLV-III is not endogenous to the human genome, and data have revealed that individual viral genomes show degrees of nucleic acid heterogeneity (*7, 8*). Virus isolation studies from patients with acquired immune deficiency syndrome (AIDS) and AIDS-related complex (ARC) and from individuals in several of the groups at high risk for the disease showed a high probability of virus presence, although HTLV-III appeared to be present in a minority of cells within an organ (*2, 8*). Antibody detection by an enzyme-linked immunosorbent assay or electrotransfer tests in individuals with AIDS or ARC and those in the high-risk groups have unambiguously defined the association of HTLV-III to AIDS (*4, 5, 10*).

Although the presence of antibody to HTLV-III in humans defines previous exposure to the virus, the presence of antibody to viral proteins does not impart immunity because advanced AIDS patients have readily detectable antibodies (*3–6, 11*). HTLV-III can be isolated from most antibody-positive subjects (*2*) and occasionally from normal-appearing, high-risk individuals who do not have detectable antibody (*12*). It therefore seems that a protected individual, that is, one with no residual virus and a strong neutralizing or other protective antibody response, has not been identified. In lieu of a simple quantal neutralization test for HTLV-III, we felt it would be advantageous to identify the HTLV-III viral envelope glycoprotein (gp) and antibodies to the glycoprotein in various virus-exposed groups because, for many viruses, the major envelope glycoprotein contains epitopes that elicit protective antibodies (*13*). The identification of the viral glycoprotein is a precondition for understanding the nature of the protective state and consequently the individual who could successfully cope with or overcome infection with HTLV-III.

A tentative description of one or more high molecular weight proteins precipitated by AIDS sera as the possible glycoprotein of HTLV-III has been made (*10*). We have identified the major viral glycoprotein and the major structural group-specific antigen together with their precursor polypeptides by a combination of immunoaffinity chromatography, radioimmunoprecipitation, and two-dimensional peptide mapping techniques. The identification of the envelope and the structural gene products was accomplished by immunoaffinity chromatography of cell culture fluids containing HTLV-III (Fig. 1). Lane 1 shows the immunoreactive polypeptides isolated from virus-containing cell culture fluids from H9 cells infected with

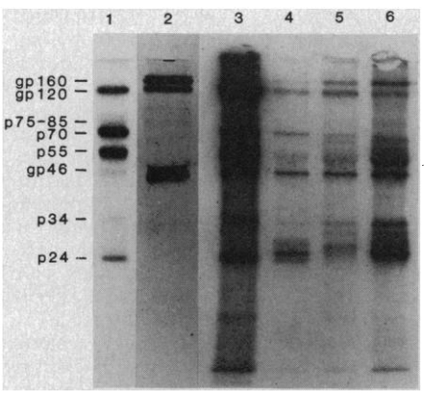

Fig. 1. Detection of HTLV-III–related antigens in extracellular extracts (lane 1) and detergent extracts (lanes 2 and 6) of HTLV-III–infected cells. Clarified cell culture fluids (lane 1) were chromatographed over AIDS IgG–Sepharose, eluted, labeled with ^{125}I by the chloramine-T method, concentrated by immunoprecipitation, and analyzed by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (*18, 21*). HTLV-III–infected H9 cells were labeled with [^{14}C]glucosamine (5 μCi/ml) for 16 hours; lysed with phosphate-buffered saline containing 1 percent Triton X-100, 0.5 percent sodium deoxycholate, and 0.1 percent sodium dodecyl sulfate; and immunoprecipitated with serum 149 after prior treatment with normal human serum (lane 2). HTLV-III–infected H9 cells were extracted with immunoaffinity buffer [1 percent Triton X-100, 1*M* KCl, and 0.01*M* tris (*p*H 8.5)] and chromatographed over AIDS IgG–Sepharose (lane 3), chimpanzee IgG–Sepharose (lane 4), AIDS IgG–Sepharose A3 (lane 5), and A4 (lane 6). The eluted material was labeled with ^{125}I as above and concentrated by immunoprecipitation with the respective homologous serum. The molecular sizes were estimated relative to the migration of the molecular weight standards myosin (200K), β-galactosidase (116K), phosphorylase B (93K), bovine albumin (68K), ovalbumin (46K), carbonic anhydrase (30K), soybean trypsin inhibitor (21K), and lysozyme (14.4K). The molecular weights assigned to gp160 and gp120 are subject to some degree of error because of the nonlinear relation of the migration of molecular weight standards in the upper range of the gel. Also, the polypeptide conventionally designated as p24 is probably somewhat heavier.

HTLV-III (*1*). The immunoaffinity resin used in this experiment was prepared from the immunoglobulin G (IgG) fraction of a serum from an AIDS patient (serum 149) that had a high reactivity to HTLV-III polypeptide p24. Several polypeptides were identified as related to HTLV-III. Cells infected with HTLV-III were also labeled with [^{14}C]glucosamine, and cell extracts were subjected to immunoprecipitation with serum 149 (Fig. 1, lane 2). The results showed that two of the previously isolated species of polypeptides, gp120 and gp46, were glycoproteins. An additional glycoprotein, gp160, was identified in cell extracts. Isotopically labeled glucosamine was not detected in the region of p75-85.

In a related experiment, detergent extracts of HTLV-III–infected cells were chromatographed over immunoaffinity resins prepared from the IgG fractions of serum 149 (Fig. 1, lane 3), serum from a chimpanzee infected with HTLV-III (*14*) (lane 4), and two sera from selected AIDS patients with Kaposi's sarcoma (lanes 5 and 6). Cytoplasmic extracts of virus-infected cells are useful for detecting virion polypeptide precursors. A prominent gp160 was detected in addition to virion gp120. At best, trace quantities of p75-85 were observed, suggesting a virion or extracellular location for this molecule. Additionally, p70, p55, p46, p34, and p24 were detected. In general, sera that were reactive with p24 (149 and chimpanzee) reacted with p70, p55, p34, and p24. The chimpanzee serum was marginally reactive with p24 and p55. In contrast, the two sera not appreciably reactive with p24 (A3 and A4) showed little or no precipitation of p70, p55, p34, or p24. However, gp160 and gp120 were readily detected. The A3 serum may precipitate a trace polypeptide in the region of p70. Additionally, the A3, A4, and chimpanzee sera did precipitate several molecules in the size range of p27 to p32 that were not detected by serum 149.

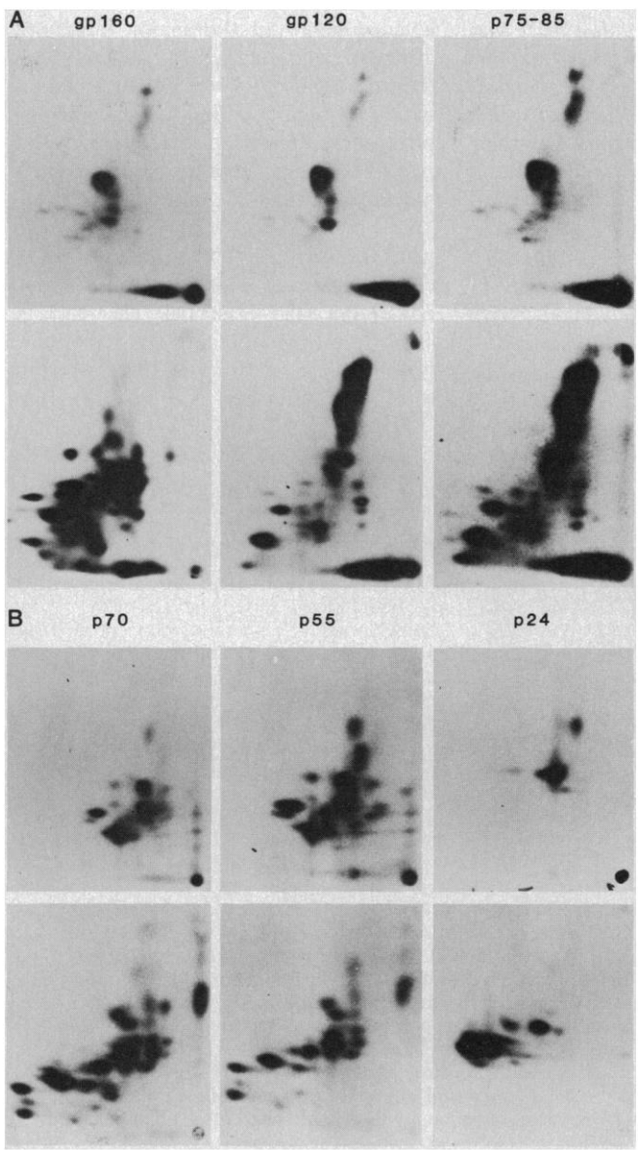

Fig. 2. Two-dimensional oligopeptide maps of HTLV-III envelope gene polypeptides (A) and structural gene polypeptides (B). Trypsin maps (upper row in A and B) and chymotrypsin maps (lower row in A and B) were made as described (*18, 21*). Briefly, polypeptides were isolated from cell culture fluids (gp120, p75-85, p70, p55, and p24) or detergent extracts of HTLV-III–infected cells (gp160) by immunoaffinity chromatography over AIDS IgG–Sepharose, labeled with ^{125}I, concentrated by immunoprecipitation, and localized on 10 percent acrylamide gels by autoradiography. Excised gel bands were digested with TPCK (L - 1 - tosylamide -2 - phenylethyl chloromethyl ketone)–trypsin or α-chymotrypsin, and resulting peptides were subjected to electrophoresis (first dimension) and ascending chromatography (second dimension). The origin for electrophoresis is in the lower right corner of each map.

Two-dimensional oligopeptide mapping was performed to determine whether these molecules reflected a possible precursor-product relation. The trypsin and chymotrypsin peptide maps (Fig. 2A) show that gp160 contains the major gp120 peptides plus substantial additional peptide information. This suggests that gp120 is derived from gp160 after a proteolytic modification. Polypeptide p75-85 showed the same amount of major peptide complexity as detected in gp120. A tentative explanation would be that the lower-weight polypeptide represents the intact peptide chain of gp120 that was deglycosylated in the virion or extracellular spaces. The complexity of the chymotrypsin map suggests that the gp160 preparation may not be homogeneous. A similar observation was made in a study of visna virus polypeptide processing (*15*). Two major peptides in gp120 and p75-85 (upper right quadrant) are present in the gp160 map as minor peptides. This is probably due to decreased iodination of those peptides as longer exposures of the maps to film revealed their presence. In any case, the two high molecular weight glycoproteins described here and by others (*10*) are related. The gp120's detected in infected cells by the three immunoaffinity resins prepared from human sera (Fig. 1) have been mapped and shown to be identical to the gp120 isolated from virions. Additionally, the group of polypeptides between p27 and p32 detected by the chimpanzee and Kaposi's sarcoma sera are related by peptide maps and appear within the p75-85, gp120, and gp160 molecules. This suggests that the low molecular weight species are likely to be degradation products of gp120 or gp160 or both and are not variants of the p24 *gag* gene product. The maps p27 and p32 appear to be more highly related to the maps of gp120 than to the maps of gp160.

The observation that sera strongly positive for p24 antibodies (serum 149) reacted with p70, p55, and p34 suggests a precursor-product relation between these molecules. The trypsin and chymotrypsin maps (Fig. 2B) support this relation. The proteins were isolated from virions and mapped as described above. The maps, especially chymotrypsin, showed a high degree of homology between p70 and p55. Further, p70 contains several peptides not found in p55, suggesting that p55 is part of p70. The p24 trypsin map is simple, but the three

peptides can be located in the structure of both p55 and p70. Likewise, the chymotrypsin map of p24 can be located in p55 and p70, although two peptides (those with the greatest electrophoretic mobility) have somewhat diminished mobilities in the form of the cleaved molecule. The largest polypeptide *gag* gene product, p70, may be cleaved to p55 and further processed to p24. Additional experiments will determine the translational control (*16*) and kinetic relation between p70 and p55.

Polypeptide p34 (Fig. 1, lane 1) is a candidate for the other cleavage product resulting from the processing of p55 to p24. Other investigators have speculatively identified a precursor of p24 in the range of 50,000 (50K) to 55K molecular weight (*3, 4, 10*). Polypeptide p70 has previously not been included in the p24 precursor scheme. A likely explanation is that the size of the cellular pool may be small, and indeed p70 is barely detectable in immunoprecipitates of metabolically labeled cell extracts but is readily iodinated. The major p24 precursor in metabolically labeled cytoplasmic extracts was p55, in agreement with other observations (*10*). In any case, our data show that p70 and p55 both contain sequences of p24.

Additional proteins invariably detected included a 46K glycoprotein (gp46) and often a 200K polypeptide (p200). Both were precipitated by various sera from noninfected subjects, such as normal human, normal rabbit, and immune rabbit. Initial peptide map data indicate that gp46 is not related to gp120. The presence of antibodies to gp41 in electrotransfer tests has been considered the most sensitive diagnostic criterion of HTLV-III infection (*4, 5*). Although gp41 has been conspicuously absent in immunoaffinity assays with the above sera, the 149, A3, and A4 sera all strongly react with p41 as determined in Western blot electrotransfers (*17*). We have occasionally detected small quantities of a nonglycosylated polypeptide (p41-43) in immunoaffinity experiments and have usually seen small amounts of it in immunoprecipitates of metabolically labeled cells. This nonglycosylated polypeptide was precipitated only by sera that strongly reacted with p24, suggesting that it is a minor intermediate in the processing of p55 to p24. Either the electrotransfer method must be highly sensitive to detect gp41, or gp41 is refractive to iodination. The identities of both gp41 and gp46 require further investigation.

These results show that the primary HTLV-III envelope gene product is a 160K glycoprotein that is processed to a 120K glycoprotein and something else. Both gp160 and gp120 appear to be subject to proteolytic (*18, 19*) and glycosidic degradation. The data also confirm that a major intermediate in the synthesis of p24 is p55 (*6, 9*) and identify another primary *gag* gene product of p70. That HTLV-III is a partially cytolytic virus in H9 cells (*1*) can account for the virus polypeptide precursors in both cytoplasmic extracts and extracellular virions. Finally, the detection of the relatively large envelope gene products of HTLV-III is consistent with the study by Gonda *et al.* (*9*), which suggested that HTLV-III may be closely related to the cytopathic visna lentivirus whose envelope glycoprotein has been reported to be gp135 with a precursor gp150 (*15*).

The identification of the major glycoprotein of HTLV-III should facilitate studies on the induction of neutralizing antibody and resolve the question of whether a state of protection can be achieved. At present, no individual exposed to HTLV-III is known to be protected. However, HTLV-III antibody-positive individuals in several candidate antibody-positive groups can be considered. These include the AIDS patients with Kaposi's sarcoma who have no opportunistic infections and relatively intact immune systems, ARC patients whose lymphadenopathy has improved, selected partners of AIDS patients who have remained well, and hemophiliacs who recently may have been exposed to inactivated virus in heat-treated factor VIII preparations. The techniques described above should clearly delineate whether patterns of antibody response to *env, gag*, or other HTLV-III gene products could indicate protection or antedate shifts in the status of the patient.

If genomic variants of HTLV-III indicate either a large number of subtypes or antigenic drift as seen in the related visna virus, then the number of glycoprotein epitopes required for the induction of immune protection may be extensive. Peptide mapping of the envelope glycoproteins of representative variant HTLV-III's will clarify this situation. The ability to isolate HTLV-III glycoproteins of various sizes could lead to attempts to induce protection against infection and seroconversion by a homologous virus or viruses in animal models such as the chimpanzee. Although extensive variations in the *env* gene restriction endonuclease pattern do not engender optimism for the successful establishment of protective immunity in humans, broadly protective group-specific and interspecies-specific neutralizing antibodies have been induced in model animal retrovirus systems by means of purified *env* gene products (*13, 20*).

W. GERARD ROBEY
NCI–Frederick Cancer Research Facility, Frederick, Maryland 21701
BIJAN SAFAI
Memorial Sloan-Kettering Cancer Center, New York 10021
STEPHEN OROSZLAN
Laboratory of Molecular Virology, Litton Bionetics, Inc., NCI–Frederick Cancer Research Facility
LARRY O. ARTHUR
Biological Products Laboratory, Program Resources, Inc., NCI–Frederick Cancer Research Facility
MATTHEW A. GONDA
Laboratory of Cell and Molecular Structure, Program Resources, Inc.
ROBERT C. GALLO
Laboratory of Tumor Cell Biology, National Cancer Institute, Bethesda, Maryland 20205
PETER J. FISCHINGER
NCI–Frederick Cancer Research Facility

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