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# Variations in Western Blot Banding Patterns of Human T-Cell Lymphotropic Virus Type III/Lymphadenopathy-Associated Virus

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**Serum samples from 27 patients infected with human T-cell lymphotropic virus type III (14 with acquired immune deficiency syndrome [AIDS] and 13 with AIDS-related complex) were examined for antibodies to viral proteins by the Western blot method and with four different commercial solid-phase enzyme-linked immunosorbent assays (ELISAs). Virus-specific bands on blots at molecular masses of 64, 55, 53, 41, 31, 24, and 17 kilodaltons were observed. Rank correlation matrices were calculated to relate the intensity of viral bands, stage of illness, and ELISA kit optical densities (ODs). Groups of bands tended to covary in intensity: p17, p24, and p55 (*gag* gene products); p53 and p64 (*pol* gene products); and p31 (*pol*/endonuclease gene product) and p41 (*env* gene product). Blots of sera from AIDS-related complex patients usually showed strong activity against all viral proteins, while those of sera from AIDS patients characteristically showed strong reactivity only at the *pol*/endonuclease and *env* bands. For one ELISA kit (Abbott Laboratories, North Chicago, Ill.), ODs correlated well with the *env* and *pol* band intensity scores, while ELISA ODs with other kits (from Litton Industries, Sunnyvale, Calif.; Electro-Nucleonics, Inc., Fairfield, N.J.; and E. I. du Pont de Nemours & Co., Inc., Wilmington, Del.) correlated closely with *gag* band intensity scores. We conclude that human T-cell lymphotropic virus type III Western blot patterns are determined by (i) viral protein processing pathways and (ii) the stage of illness of the patient and may reflect (iii) the ELISA method used for serum screening.**

The human T-cell lymphotropic virus type III (HTLV-III) is now clearly established as the primary etiology of the acquired immune deficiency syndrome (AIDS) and AIDS-related complex (ARC). Although the virus can be isolated regularly from the blood of infected individuals (3), the procedure is prohibitively complex and expensive for use as a routine diagnostic method. An alternative approach is the detection of virus-specific antibodies as a marker of infection (5, 9).

The genome of HTLV-III codes for the three structural proteins typical of retroviruses: the *env* gene codes for the virion surface glycoproteins, the *gag* gene codes for the virion code antigens, and the *pol* gene codes for protease, reverse transcriptase, and endonuclease activities. In addition, the HTLV-III genome codes for at least three other unique proteins (6). These gene products may undergo posttranslational modifications, e.g., cleavage into two or more smaller proteins.

The Western blot technique has been recommended as the procedure of choice for confirmation of the presence of HTLV-III antibodies in a sample of serum or plasma (9, 10). In the HTLV-III Western blot procedure, individual viral proteins are separated from contaminating cellular proteins, and from one another, into discrete bands by gel electrophoresis. The protein bands are in turn electroblotted onto the surface of a nitrocellulose paper strip, where they can be conveniently exposed to serum antibodies (12).

We observed a wide variety of HTLV-III Western blot band patterns by using different patient sera. In this study we sought to determine (i) whether the banding pattern variations observed with HTLV-III immune serum reflected virus protein processing pathways, (ii) whether the patterns differed by the stage of illness of the patient, and (iii) whether commercial HTLV-III enzyme-linked immunosorbent assay (ELISA) test kits showed differential sensitivities for antibodies directed against the various viral gene products.

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## MATERIALS AND METHODS

**Reference panel.** The reference panel consisted of 40 serum samples. Normal samples were obtained from low-risk hospital workers and were negative for HTLV-III antibodies by four commercial ELISA kits. Thirteen ARC samples were obtained from patients with clinical diagnoses of ARC as previously defined (7), representing patients with Walter Reed HTLV-III disease stages 3 to 5 (8). Fourteen AIDS samples were obtained from patients with AIDS, all of whom had severely depressed levels of T helper cells and had opportunistic infections (Walter Reed stage 6); none had Kaposi's sarcoma. Peripheral blood mononuclear cells from all 40 subjects were cultured for HTLV-III/lymphadenopathy-associated virus in the laboratory of R. Gallo (3). All normal donors were negative for virus isolation, whereas all ARC and AIDS donors were positive on at least one occasion.

**ELISAs.** Licensed commercial solid-phase ELISA kits for detection of antibodies to HTLV-III were obtained from Abbott Laboratories, North Chicago, Ill.; Electro-Nucleonics, Inc., Fairfield, N.J.; Litton Industries, Sunnyvale, Calif.; and E. I. du Pont de Nemours & Co., Inc., Wilmington, Del. All kit assay tests were performed in duplicate at the Walter Reed Army Institute of Research exactly according to the instructions of the manufacturers by using manufacturer-provided apparatus for dispensing, washing, and reading. Results were expressed as optical density (OD) values.

**HTLV-III Western blots.** Western blots were performed at Biotech Research Laboratories, Inc. The technique involved the application of 60 μg of partially purified (single banded) virions to each 12% polyacrylamide gel. Separated antigens were transferred onto nitrocellulose with 30 V for 16 h, and

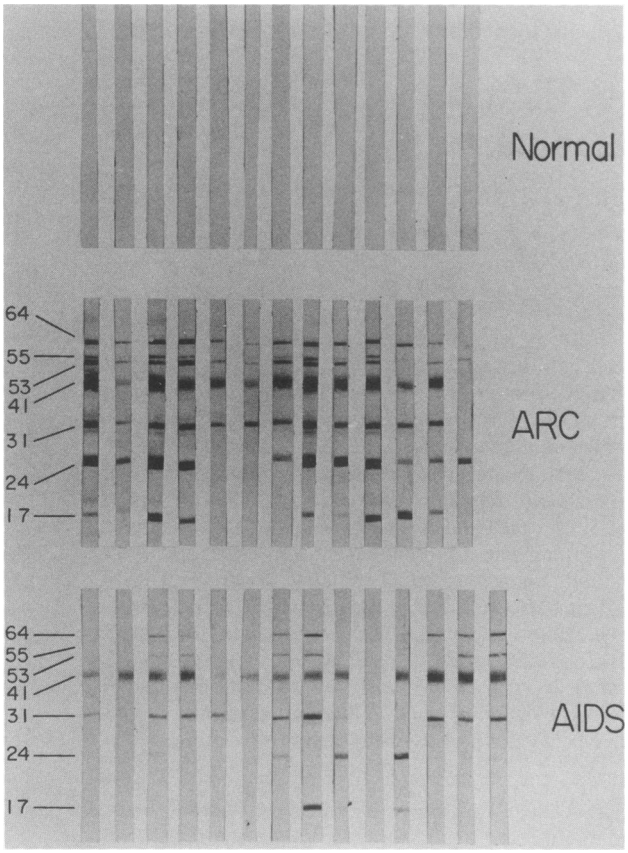


FIG. 1. HTLV-III Western blots with sera from normal uninfected subjects, patients with ARC, and patients with AIDS. Molecular masses of the major bands are shown on the left in kilodaltons.

the nitrocellulose was cut into 25 strips. Individual strips were reacted with a 1:100 dilution of test sample. Bound antibody was detected with biotin-containing goat anti-human immunoglobulin G, horseradish peroxidase-labeled avidin, hydrogen peroxide, and 4-chloro-1-naphthol as the substrate (S. S. Alexander, Jr., C.-C. Tai, R. L. Ting, A. E. Corrigan, A. J. Bodner, and D. W. Julien, Abstr. Annu. Meet. Am. Soc. Microbiol. 1985, T49, p. 297). Backgrounds were clear white, and bands were bluish purple. All specimens were tested in random order on strips from two identical gels prepared on the same day. Blots were read blindly, and band intensities were arbitrarily scored as negative, weak, definite, or strong, with corresponding values of 0, 0.5, 1, or 2 assigned for statistical analyses.

**Statistical analyses.** Stage of illness, blot band intensity scores, and ELISA OD values were correlated by calculating a rank correlation matrix with a MINITAB statistics package (Pennsylvania State University, University Park).

TABLE 1. Rank correlation matrix of HTLV-III Western blot band intensity scores

| Protein | Correlation (*r*) | | | | | | |
|---|---|---|---|---|---|---|---|
| | p64 | p55 | p53 | p41 | p31 | p24 | p17 |
| p64 | 1.00 | | | | | | |
| p55 | 0.610 | 1.00 | | | | | |
| p53 | 0.877 | 0.577 | 1.00 | | | | |
| p41 | 0.329 | 0.081 | 0.257 | 1.00 | | | |
| p31 | 0.612 | 0.339 | 0.544 | 0.661 | 1.00 | | |
| p24 | 0.392 | 0.673 | 0.315 | −0.008 | 0.170 | 1.00 | |
| p17 | 0.618 | 0.821 | 0.497 | −0.008 | 0.220 | 0.654 | 1.00 |

## RESULTS

**Western blot and ELISA results.** All 13 of the sera from normal donors yielded blank lanes when tested by Western blot, whereas all 27 sera from HTLV-III-infected patients produced multiple bands of various patterns. Virus-specific bands were observed at molecular masses of 64, 55, 53, 41, 31, 24, and 17 kilodaltons (kDa) (Fig. 1). All four ELISA kits correctly differentiated between sera from infected and noninfected patients.

**Correlations between Western blot bands.** The rank correlation matrix derived by using band intensity scores from blots of the 27 HTLV-III-infected patients is shown in Table

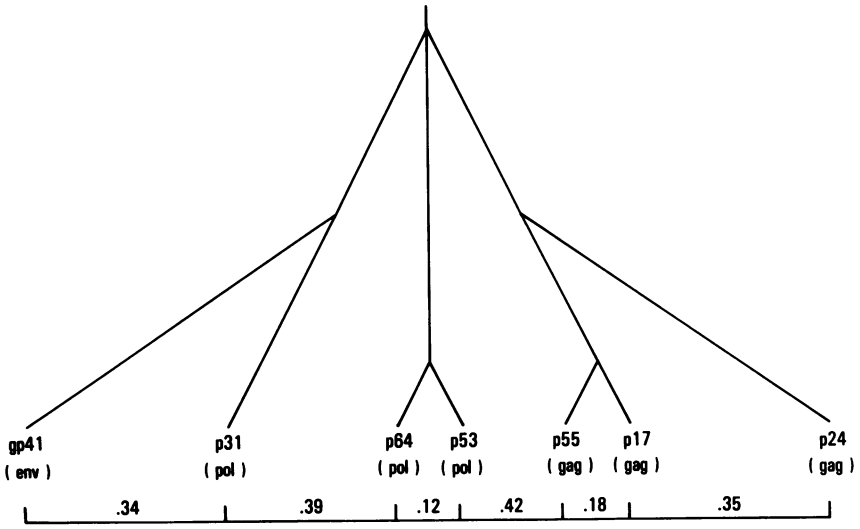


FIG. 2. Dendrogram schematic representation of HTLV-III antibody correlations. Numbers on the line below the dendrogram are $1 - r$, where $r$ is the rank correlation coefficients of band intensitites of most closely related pairs.

TABLE 2. Western blot band intensity scores of patients with AIDS or ARC and healthy normal controls

| Group | Band intensity[a] | | | | | | |
|---|---|---|---|---|---|---|---|
| | p17 | p24 | p31 | p41 | p53 | p55 | p64 |
| Normal ($n$ = 13) | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| ARC ($n$ = 13) | 1.2 ± 0.8 | 1.7 ± 0.7 | 1.6 ± 0.7 | 1.8 ± 0.6 | 1.4 ± 0.7 | 1.0 ± 0.8 | 1.6 ± 0.6 |
| AIDS ($n$ = 14) | 0.2 ± 0.5 | 0.4 ± 0.7 | 1.3 ± 0.0 | 1.7 ± 0.5 | 0.6 ± 0.7 | 0.1 ± 0.3 | 0.7 ± 0.7 |

[a] Mean ± 1 standard deviation.

1. The strongest correlations were between antibodies to p64 and p53 ($r$ = 0.877) and between antibodies to p17 and p55 ($r$ = 0.821), while the weakest correlations were between antibodies to p41 and p24 or p41 and p17 (for both correlations, $r$ = −0.008). Antibodies to p41 correlated well only with those to p31 ($r$ = 0.661), while antibodies to p31 also correlated well with those to p64 ($r$ = 0.0612) and p53 ($r$ = 0.544). Antibodies to p24 correlated well with those to p17 ($r$ = 0.654) and p55 ($r$ = 0.673). A dendrogram (Fig. 2) depicts the relatedness of antibody responses to the seven viral proteins.

**Correlation of stage of illness with antibody response to individual viral bands.** Western blot band intensity scores in patients with AIDS and ARC are shown in Table 2. The p41 band intensity did not vary appreciably with the stage of illness, whereas the bands at p55, p17, and p24 were markedly diminished in blots of sera from AIDS patients compared with those from ARC patients.

**Correlation of commercial ELISA kit results with antibody response to individual viral bands.** The rank correlation matrix derived by using commercial solid-phase ELISA kits and band intensity scores is shown in Table 3. The Abbott ELISA kit ODs covaried predominantly with the band intensities at p31, p53, p64, and p41. All three other ELISA kit ODs covaried predominantly with the band intensities at p17, p24, and p55. Because band correlations with the Abbott kit differed substantially from those of the other kits, the samples were retested with the Abbott kit. The initial observations were verified.

**Rank correlations of the stage of illness with ELISA kit OD values.** Rank correlation coefficients of stage of illness with ELISA kit OD values were as follows: Abbott, −0.374; Litton, −0.491; Electro-Nucleonics, −0.640; and du Pont, −0.733.

## DISCUSSION

The exact genomic origins of several of the HTLV-III antigens have been clearly established (2). The *gag* gene codes for a 55-kDa polyprotein (p55) which is cleaved into three smaller proteins: p24 (middle), p15 (carboxy terminus), and p17 (amino terminus). The p15 protein is thought to undergo further proteolytic cleavage. The *env* gene encodes for a 90-kDa protein which is richly glycosylated to yield a 160-kDa glycoprotein, which in turn is cleaved into a 120-kDa amino terminus external viral membrane glycoprotein and a 41-kDa carboxy terminus transmembrane glycoprotein. The precise protein processing of the *pol* gene is less well defined. However, both p64 and p53 have recently been shown to be *pol* gene products by comparison of the amino-terminal peptide sequences of the proteins with the *pol* gene nucleotide sequence, and purified p64/p53 has reverse transcriptase activity (13). (Note that the *pol* gene product molecular weights are referred to as p66/p51 by Veronese et al.) p31 has also been identified as a product of the carboxyl region of the *pol* gene. Bacterial transformants expressing the putative endonuclease domain of the *pol* gene selectively competitively inhibit binding of the sera of AIDS patients to p31 (11). Products of the other known HTLV-III genes (*sor*, 3′-*orf*, and *tat*), are nonstructural, intracellular proteins, which we could not detect by Western blots with concentrated virus. However, antibodies to these gene products can be detected in human sera by using other techniques (1, 4). A diagram of the genomic origins of HTLV-III antigens is shown in Fig. 3.

Our data demonstrate that the serum from an HTLV-III patient, if able to recognize one of the *gag*-derived proteins, tends to recognize all three proteins (p55, p24, and p17). In this respect, the Western blot pattern reflects the protein processing of *gag* gene products. Similarly, the extremely close correlation of the p64 and p53 band intensities in Western blot reactions with human antibodies reflects the processing of the mid-*pol* gene product. p31 band intensities correlated equally well, but loosely, with those of the *env*-derived p41 and those of mid-*pol*-derived p64.

Another factor associated with the Western blot pattern is the stage of the illness of the patient from whom the test serum sample was drawn. We observed, as have others, that the intensity of *gag*-derived bands was markedly less with sera from AIDS patients compared with sera from ARC patients (10), while in a similar comparison intensities of the *env*-derived p41 bands were nearly equal for both stages of illness. *pol* band intensities (p64, p53, and p31) were diminished in AIDS sera, but not to the same degree as the *gag*-derived proteins. These results suggest that it may be possible to "stage" a patient based on the ratio of the serum antibody activity against *gag* and *env* proteins; early in the

TABLE 3. Rank correlation matrix of ELISA kit results with Western blot band intensity scores

| ELISA kit | Correlation ($r$) | | | | | | |
|---|---|---|---|---|---|---|---|
| | p41 | p31 | p64 | p53 | p55 | p17 | p24 |
| Abbott | 0.607 | 0.777 | 0.659 | 0.680 | 0.424 | 0.357 | 0.270 |
| Litton | 0.269 | 0.146 | 0.359 | 0.405 | 0.384 | 0.286 | 0.470 |
| Electro-Nucleonics | 0.303 | 0.541 | 0.467 | 0.458 | 0.692 | 0.526 | 0.659 |
| du Pont | 0.112 | 0.340 | 0.566 | 0.446 | 0.805 | 0.802 | 0.768 |

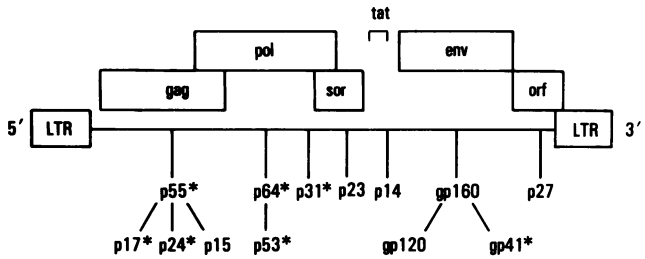


FIG. 3. Schematic representation of HTLV-III genome structure and known gene products. Proteins detectable by Western blot are indicated by asterisks.

course, the p24/p41 antibody ratio is high, and in the late or terminal stages the ratio is low.

Sensitivities of commercial ELISA kits for detection of antibodies to individual viral proteins differed substantially. Abbott ELISA kit OD values covaried predominantly with the intensities of the *pol* and *env* Western blot bands, while with other kits, most notably that manufactured by du Pont, ELISA OD values covaried predominantly with the band intensities of the *gag* gene products. As a consequence, Abbott ELISA kit OD values were not appreciably different when ARC and AIDS sera were compared, while du Pont ELISA kit OD values were substantially higher with ARC compared with AIDS sera. It follows that if the du Pont ELISA preferentially detects *gag* antibodies, then blots performed on sera deemed positive by this assay are more likely to show relatively intense banding patterns at p55, p24, and p17. However, this contention remains to be proven in large-scale screening programs.

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