CONCISE REPORT

Immunogenic Nature of a Pol Gene Product of HTLV-III/LAV


The present studies were initiated to define the coding region of a 34 kilodalton (kd) protein (p34) frequently observed with antibodies from HTLV-III/LAV-infected people by immunoblotting and radioimmunoprecipitation (RIP) techniques. We have directly mapped this viral protein to the pol gene of HTLV-III/LAV by radiolabeled amino acid sequence analysis. This region at the 3' end of the pol gene is predicted to encode the endonuclease/integrase of the virus. The seroprevalence rate of antibodies to the pol gene products p64 and p53 and to the endonuclease p34 were evaluated. Of 161 HTLV-III/LAV seropositive people tested by immunoblotting procedures, greater than 98% had antibodies which reacted to p64/p53 and 92.6% reacted to p34 indicating that these viral proteins are highly immunogenic in nature. We have also analyzed the serum of nine healthy people living in West Africa who were infected with HTLV-IV, a closely related retrovirus. Nine of nine seropositive people had antibodies that cross-reacted to p34 of HTLV-III/LAV, whereas only seven of nine reacted to p64/p53. These studies and our earlier observations indicate that current diagnostic procedures for screening for HTLV-III/LAV infection may also detect HTLV-IV seropositive individuals, pointing to a need for more specific assay systems.

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RESULTS

An example of the serum profiles obtained by immunoblotting procedures is shown in Fig 1A. Represented is a healthy laboratory HTLV-III/LAV seronegative person (lane 1), two healthy homosexual seronegatives (lanes 2 and 3), three healthy HTLV-III/LAV seropositives (lanes 4 through 6), three AIDS-related complex (ARC) patients (lanes 7 through 9), and three AIDS patients (lanes 10 and 11).

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through 12). Serum from all seropositive individuals recognize a 34-kd protein (p34). A comparison with RIP/SDS-PAGE is shown in Fig 1B. In every seropositive case shown, a p34 species was observed using this procedure.

To ascertain whether this protein was encoded by the genome of HTLV-III/LAV, radiolabeled sequence analysis was performed. p34 was individually radiolabeled with [3-H] phenylalanine, isoleucine, and lysine, isolated by SDS-PAGE, and subjected to amino-terminal amino acid sequencing. The results are shown in Fig 2. Phenylalanine peaks were observed at positions 1 and 26, lysine peaks were seen at 7 and 14, and an isoleucine peak was seen at position 5 of the p34 sequence. Analysis of the predicted amino acid sequence from the pol gene region indicates that p34 maps to the 3' end of this gene and begins at nucleotide sequence 4263 of the HTLV-III/LAV genome as published by Ratner et al. The predicted amino acid sequence is shown in Fig 2B. The probability of a protein with a random amino acid sequence having lysines, phenylalanines, and an isoleucine in these positions is less than $5.5 \times 10^{-5}$.

We analyzed 205 serum samples from people at high risk for infection and those with evidence of HTLV-III/LAV-related disease for the seroprevalence of p34 (Table 1). Of these samples, 161 were positive for antibodies to HTLV-III/LAV proteins by immunoblotting. Overall, 92.6% of seropositives had antibodies to p34, whereas the seroprevalence ratio to p64/p53 was greater than 98%, higher than any other category of protein including the env-encoded gp41. It should be noted, however, that all seropositives detected gpl20 and gpl60 by RIP/SDS-PAGE procedures. Prevalence of antibodies to reverse transcriptase p64/p53 is high irrespective of disease state, but the prevalence to p34 drops slightly in the AIDS category. Additionally, we examined nine representative serum samples from HTLV-IV-
infected people for antibodies to the pol gene products of HTLV-III/LAV. Three serum profiles are illustrated in Fig 1A, lanes 13 through 15. In addition to reactivity to the gag-related antigen p24 of HTLV-III/LAV, all nine samples recognized the p34 protein whereas only seven of nine detected p64.

**DISCUSSION**

As each new viral protein has been identified and its seroprevalence assessed, a better understanding of the humoral response to infection unfolds. The gene products can then be evaluated on the basis of their relative antigenicity. The present results indicate that the pol gene products which include the previously identified p64/p53 and the above-discussed endonuclease p34 are highly immunogenic in HTLV-III/LAV–infected people. The most immunogenic proteins are the env-encoded glycoproteins gp160 and gp120, followed by pol-encoded p64/p53, env-encoded gp41, gag-encoded p24, and pol-derived p34. The relationship between serologic profiles and disease state has received much attention from investigators in hopes of finding key serologic markers indicative of clinical progression of disease. Unfortunately, no clear pattern has emerged although relative decreases in seroprevalence of p24, p17, and p34 have been observed in AIDS patients.

Perhaps the most intriguing aspect of the antigenic nature of these viral proteins relates to the high degree of immunologic cross-reactivity between HTLV-III/LAV and HTLV-IV. The significance of these findings is obvious when the design of second-generation screening assays is devised for detecting antibodies to HTLV-III/LAV. As previously reported, many HTLV-IV–infected people test positive by enzyme-linked immunosorbent assay (ELISA) to HTLV-III/LAV. The exact nature of this ELISA cross-reactivity is unknown although limited cross-reactivity exists between env-encoded proteins for these viruses as well as the gag- and pol-derived proteins. Detailed analysis of cross-reactive epitopes is necessary, therefore, to devise assays that would include or exclude conserved epitopes. In the former case, inclusion of conserved epitopes would be helpful for screening for HTLV-III/LAV and related viruses in blood banking situations. In the latter case, nonconserved epitopes in an assay system would be preferred for delineating between HTLV-III/LAV and HTLV-IV exposure, which may then have clinical relevance.

**REFERENCES**

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