Spectrum of Natural Antibodies Against Five HTLV-III Antigens in Infected Individuals: Correlation of Antibody Prevalence With Clinical Status

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The genome of the HTLV-III/LAV retrovirus, the etiologic agent of the acquired immunodeficiency syndrome (AIDS), encodes the viral structural proteins (envelope and core proteins), the reverse transcriptase, a transactivation protein (tat-III), as well as two other proteins (3'orf, sor) of unknown function. We studied the prevalence of natural antibodies against envelope, gag, 3'orf, sor, and tat-III in the sera of HTLV-III infected individuals in an attempt to correlate clinical status with seropositivity to specific HTLV-III antigens. We selected 101 sera; 16 were obtained from normal donors with no known risk factors, and 85 were from patients with full-fledged AIDS (28 cases), AIDS-related complex (ARC, 22 cases), and healthy people at risk (homosexuals, intravenous [IV] drug users, relatives of AIDS patients; 35 cases). Seropositivity for antibodies against the envelope (gp41) and gag antigens (p15, p24) was determined by Western blot using disrupted HTLV-III virions. Of the 101 sera, all 16 from nonrisk donors and 3/35 from healthy at-risk donors were negative for antibodies against either the gp41 or p15 and p24. The remaining 82 sera were seropositive for either the gp41 and/or the p15 and p24. All sera were then tested against the three known HTLV-III antigens (3'orf, sor, and tat-III) that have been synthesized in bacteria. Our data indicate that all the HTLV-III antigens tested are immunogenic in vivo. No significant difference in antibody prevalence to gp41 (close to 100%) and to the 3'orf, sor, and tat-III proteins (approximately 50%) was observed with regard to stage of the disease. In contrast, the prevalence of antibodies against the core antigens decreased from approximately 100% in infected people with no clinical signs of disease to 50% in ARC and AIDS patients. The percentage of patients seropositive for all five antigens tested was increased in the AIDS group. These results indicate that the greatest antibody prevalence was obtained using viral envelope antigen and further suggest that screening with the newly identified 3'orf, sor, and tat-III proteins as antigens would confer no further diagnostic advantage. The pattern of natural antibodies observed during disease progression did not suggest any pathogenetic mechanism.

Aquired immunodeficiency syndrome (AIDS) was first described in the United States in 1981, and since then the disease has reached epidemic proportions. The syndrome consists of a series of clinical manifestations directly related to the severe immune dysfunction in the affected people, such as opportunistic infections and various disorders such as autoimmune diseases, malignancies, and neurologic manifestations, the pathogenic origin of which is less obvious. Infection by HTLV-III/LAV is the common denominator among these patients. HTLV-III infection correlates with depletion of the OKT4+ T cells in vivo and in vitro. While the immunodepression observed in patients with AIDS could be a direct consequence of the cytopathic effect of HTLV-III exerted on T cells as it is observed in vitro, the role played by HTLV-III in the pathogenesis of the various clinical manifestations of the syndrome is still not clear. The molecular cloning and the nucleotide sequencing of HTLV-III allowed the identification of the envelope, polymerase and the core protein genes, and three additional open-reading frames, 3'orf, sor, and tat-III, that encode proteins of 27, 23, and 14Kd respectively. The tat-III protein seems to enhance virus expression in the infected cells and may play a key role in the mechanism of viral replication. The functions of the 3'orf and sor proteins relative to the viral life cycle or the cellular metabolism of infected cells remain to be uncovered. We studied the expression in vivo of these viral genes by analyzing the natural antibody response in infected individuals in an attempt to correlate the stage of the disease and the different clinical manifestations with the expression of specific viral proteins.

MATERIALS AND METHODS

Virus preparation. HTLV-III produced by H9 cells was harvested from 50 liters of cell-free tissue culture media using zonal ultracentrifugation on two sequential ribonuclease-free sucrose gra-

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0006-4971/87/6902-0008$3.00/0

exchange Water Protein-Pak Sp-5PW HPLC column and elution with a 25-mL gradient of 0% to 80% 0.75 mol/L NaCl in the equilibration buffer. The bacterial sor (p18) is a hybrid protein representing 83% of the sor gene. The native protein product has been identified as a 23kD protein. The tat-III protein was purified by applying the bacterial lysate to a 15% SDS-polyacrylamide gel. After electrophoresis the 14 Kd tat-III protein expressed in *Escherichia coli* is not a fusion protein but represents the complete protein product of the tat-III gene. It therefore has the same size as the native protein product detected in infected cells.

**Western blot analysis of the viral proteins.** Lysate of HTLV-III or purified recombinant proteins (3’orf, tat-III, and sor) were fractionated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). The proteins were electrothermally transferred to nitrocellulose sheets as described, and the sheets were incubated for one hour at room temperature with a solution containing 5% nonfat dry milk and 0.01% Antifoam (Sigma, St Louis) in PBS to block nonspecific protein-binding sites. Strips were obtained from the nitrocellulose sheets and individually reacted with human sera diluted 1:100 for one hour at 4°C in the same solution containing 5% nonfat dry milk. After incubation the strips were washed in PBS containing 0.5% sodium deoxycholate and reacted for 20 minutes with 125I staphylococcal protein A (5 × 10^5 cpm/mL). The strips were washed again, and autoradiograms were obtained.

**RESULTS**

**Location of the coding regions for the HTLV-III proteins.** A summary of the genes identified in the different open-reading frames of the HTLV-III genome by computer analysis is depicted in the upper part of Fig 1. To date the core proteins p24, p15, have been identified as well as the gp120 and gp41 for the exterior and transmembrane portion of the envelope respectively. Three other HTLV-III genes have been uncovered by studying the viral c-DNAs, and the features of their spliced mRNAs are summarized in the lower part of Fig 1. The native products of the three genes, 3’orf, sor, and tat-III have been identified in infected cells cultured in vitro. The three genes encode proteins p27, p23, and p14 respectively.

**Fig 1.** Diagrammatic representation of the HTLV-III genome and of the spliced mRNAs for three protein products. The upper part of Fig 1 displays the HTLV-III genome and the position of the open-reading frames for the six genes that have been identified. The schemes underneath the HTLV-III map include the sequences that are maintained in the mature mRNAs for the 3’orf, sor, and tat-III proteins. The white and black dots represent the donor and acceptor sites that are joined together in the mature mRNA. The S’ donor splice site for the sor mRNA has not been identified yet. The numbers refer to the position of the sites in the HTLV-III nucleotide sequence.

**Correlation of antibody response and stage of the disease.** We attempted to correlate the stage of the disease with the prevalence of antibodies against specific viral antigens. We employed 82 human sera seropositive for antibodies against the HTLV-III core proteins (p15 and/or p24) or the gp41 or both. The sera, all obtained from Americans, included 32 samples from healthy individuals at risk for HTLV-III infection; 28 samples from patients with full-fledged AIDS, and 22 samples from American patients with ARC. The patients in the risk group defined 'healthy' included individuals with and without lymphadenopathy (LAS). Among them, three were IV drug users and one was a relative of a patient with AIDS. The rest were homosexuals.

**Table 1.** Seroreactivity of sera from patients with HTLV-III infection to bacterially synthesized proteins. The sera, all obtained from Americans, included 32 samples from healthy individuals at risk for HTLV-III infection; 28 samples from patients with full-fledged AIDS, and 22 samples from American patients with ARC. The patients in the risk group defined 'healthy' included individuals with and without lymphadenopathy (LAS). Among them, three were IV drug users and one was a relative of a patient with AIDS. The rest were homosexuals.

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sor protein. This reactivity was specific for the recombinant protein, since a protein of similar size is not detected in the control bacterial lysate (Fig 4, lanes 1 and 2). Similarly, the specific antiserum obtained from a rabbit immunized with the purified protein reacted with the recombinant sor protein (lane 4), while no reaction was observed in the total bacterial lysate (lane 3). The sor protein used in these studies is a chimeric protein that contains at the amino terminus 13 amino acids derived from the p21E gene of the vector. To rule out that the reactivity of the human sera was directed against vector epitopes rather than sor epitopes, we tested the reactivity of the same sera against another chimeric protein expressed in the same vector containing 27% of the coding sequences of the HTLV-I p21E gene. Figure 4, lanes 5 and 6, shows that a human serum positive for the recombinant sor protein does not recognize the p21E recombinant HTLV-I chimeric protein, while a serum obtained from a patient with adult T cell leukemia does. Thus, the reactivity of human sera from uninfected individuals seems to be directed against the antigenic determinants of the recombinant sor protein.

**DISCUSSION**

All the known proteins encoded by HTLV-III are immunogenic in vivo, but the spectrum of antibodies that they
elicit in infected individuals does not seem to correlate with disease stages as they are currently described. We noticed that as infected individuals progressed toward AIDS, they tended to acquire antibodies to all the HTLV-III antigens studied (data not shown). This finding may simply reflect continual expression of the virus in vivo with a greater likelihood of exposure to each antigen with increasing time after infection. Fluctuations in antibody prevalence were observed with stage of disease, particularly with the sor and tat-III proteins, although the differences observed did not yield statistically significant correlations. These varying prevalences may result from different levels of circulating antigens with disease progression and immune complex formation, leading to lessened detection of serum antibodies.

More extensive studies would be necessary to determine whether the observed fluctuations have underlying biologic significance. Direct analysis for circulating immune complexes or complexes trapped in lymph nodes would be informative. It is remarkable that the immune response against all HTLV-III antigens in AIDS patients seems to be comparable to that of the healthy at-risk patients, despite the high degree of immune system failure observed in the former group. Our data suggest that it may be necessary to investigate individuals very soon after viral infection to determine whether sequential exposure to HTLV-III antigens is correlated with laboratory or clinical manifestations of AIDS. In this regard we analyzed sera of three patients at risk who were virus positive, seronegative for antibodies against the HTLV-III envelope and gag proteins and who subsequently seroconverted. Their sera were negative for the 3'orf, sor, and tat-III before the time of seroconversion, suggesting that these proteins might not be useful in the diagnosis of the early phase of infection. More serum samples from this kind of patient should be studied before a definitive conclusion on this point can be reached. Our analysis has shown that of the five HTLV-III antigens studied, the most immunogenic one appears to be the gp41 transmembrane protein. As the major envelope protein gp120 also appears to be highly immunogenic, it would be useful to include it in screening procedures when the purified protein becomes available. Obviously the use of a combination of bacterially synthesized antigens for large scale antibody testing not only could increase the sensitivity of antibody detection but could also eliminate the problem of obtaining false-positive results due to contamination of virus preparations with human antigens.

In this regard it should be noticed that the sor protein should not be used in the antibody screening procedure because it could be the cause of false-positive results. In fact, we have shown that some human sera from not-at-risk, uninfected individuals have antibodies that react with the sor antigenic determinants. We believe that this reactivity may be due to crossreactive determinants in either endogenous or exogenous proteins that elicit an immune response in humans.

ACKNOWLEDGMENT

We are very grateful to Drs J. Ghrayeb, T. Papas, C. Debouck, and K. Samuel for providing purified proteins and to Dr Robert C. Gallo for his support. We also thank Dee Goodrich for editorial assistance.

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