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Antibody to Human Endogenous Retrovirus Peptide in Urine of Human Immunodeficiency Virus Type 1-Positive Patients

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Human endogenous retrovirus (HERV)-like sequences are normal inherited elements that constitute several hundredths of the human genome. The expression of genes located within these elements can occur as a consequence of several different events, including persistent inflammation or genotoxic events. Antibodies to endogenous retroviral gene products have been found in a number of infectious, chronic, and malignant diseases, suggesting a role in disease initiation and progression. We studied human immunodeficiency virus type 1 (HIV-1)-infected patients for evidence of urine antibody to a HERV peptide and investigated correlates with clinical and laboratory parameters. Forty-three HIV-1-infected patients in documented asymptomatic, symptomatic, or AIDS stages of disease and 21 age- and gender-matched, uninfected controls were tested for antibody to HERV-related peptide 4.1. Urine specimens were examined in a blinded fashion with the Calypte Biomedical Corp. experimental enzyme immunoassay for antibody to peptide 4.1. Results were compared with demographic data, medical history, clinical state of disease, and results of other laboratory tests. Thirty-six percent of the asymptomatic (Centers for Disease Control and Prevention [CDC] category A) and 81.3% of both the symptomatic (CDC category B) and AIDS (CDC category C) patients were positive for antibody to HERV-related peptide 4.1. None of the controls were positive. In this study, antibodies to HERV-related peptide 4.1 were found more frequently in patients with advanced stages (categories B and C) of HIV-1 disease than in those patients with an earlier stage (category A) of HIV disease. In HIV patients, severe immunosuppression, defined as having had at least one opportunistic infection, correlated with the expression of antibody to a HERV-related peptide.

Endogenous retrovirus-like elements (ERVs) are transposable genetic elements in eukaryotes that structurally resemble retroviruses and use RNA intermediates in their replicative cycles. ERVs are normal inherited genetic elements found in all mammals (11, 18, 26, 29). In the human genome, there are many families of ERVs, most consisting of multiple copies of the element. They may be divided into two groups based upon the presence or absence of long terminal repeats (LTRs). Those with LTRs can be further divided based on infectivity. Infectious elements with LTRs are retroviruses, while noninfectious elements with LTRs are retrotransposons (18). ERVs lacking LTRs are called retroposons. Together, these human ERVs (HERVs) comprise several hundredths of the total genome.

Although infectious endogenous retroviruses have been identified in nonhuman species, all HERVs that have been identified to date appear to be noninfectious because of structural defects. Nevertheless, these HERVs may alter the expression of cellular genes via transposition into or near the genes or through the activity of transcriptional regulatory sequences found in the HERV LTRs.

There is considerable evidence for the expression of HERV genes in human cells (17, 29). However, the mechanisms responsible for the expression of these genes are not clearly understood. In general, it is known that inflammatory responses induced by injury, toxic chemical agents, radiation, or infectious agents contribute to the activation and expression of genes found on transposable genetic elements (6). More specifically, sequences within short interspersed element DNA, or *Alu* sequences, are activated by human immunodeficiency virus type 1 (HIV-1) infection (16). Moreover, activation and *Alu*-mediated recombination are recognized as contributing factors in the progression of some chronic disorders (27). In addition, with the development of xenogenic therapies for human diseases, there is the potential for nonhuman retroelements to be unintentionally incorporated into the human genome. The effects that these elements may have on human cells are of increasing concern (13).

The detection of antibodies to proteins encoded by genes found on HERVs has been reported for a number of infectious, chronic, and malignant diseases (1a, 3, 21). The presence of these antibodies indicates that the immune system has been activated, suggesting that there is a retroelement-associated influence on the immune system. It is therefore possible that disease progression can be predicted by the detection of antibodies to HERV-specific antigens (18, 26).

Retrovirus-like elements of interest in the study of human diseases include those presumably derived from bovine, feline, murine, and human leukemia viruses (18, 26). Provirus and retrotransposon precursors of these viruses produce a conserved transmembrane envelope protein with a molecular mass of approximately 15 kDa, p15E, which is involved in the suppression of cell-mediated immunity (14, 23, 24). CKS-17A is a 17-amino-acid peptide derived from p15E. It has been shown

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to inhibit lymphocyte proliferation and to modulate T-helper 1 and T-helper 2 cell responses (7, 12, 15). For this study, peptide 4.1, a 17-amino-acid synthetic peptide partially homologous to CKS-17A, was constructed from p15E amino acid sequence data.

Antibodies against HERV gene products can be detected in various body fluids, including blood, urine, and oral fluids. A recent evaluation of the results of a large clinical study showed that there was a compartmentalized response to the exogenous retrovirus HIV-1. Antibodies were found in urine but not in blood (25, 28). The data from that study on urine anti-HERV antibodies and the results on anti-HIV antibody compartmentalization suggest that, of these two, the presence of antibodies against HERV gene products in urine may be a more sensitive indicator of an active immune response (8, 19).

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MATERIALS AND METHODS

Human subjects. Forty-three HIV-positive patients from the Infectious Disease Clinic of the Stratton VA Medical Center were enrolled in this study from August 1995 to March 1996. All patients were tested and found to be positive for anti-HIV antibodies by the Wadsworth Laboratories of the New York State Department of Health, Albany. The control subjects were 21 age-matched, healthy male volunteers with no known risk factors for HIV infection.

Urine specimens. At the time of the clinic visit and after signing a previously approved Institutional Review Board consent form, each patient provided one urine specimen collected sometime between late morning and early afternoon (hospital specimen). Thereafter, over a 2-week period, each patient provided four urine specimens obtained at home. One was obtained in the early morning, one at noon, one in the late afternoon, and one in the evening. These specimens, collectively referred to as home urine specimens, were collected at different times of day in case there was diurnal variation in the level of urine anti-HERV antibodies. The same specimen collection protocol was followed for control subjects. Urine specimens were preserved with Stabilar preservation tablets (RP Cargille Laboratories, Inc., Cedar Grove, N.J.), stored at room temperature (15 to 30°C) for 1 to 15 days prior to initial testing at the Stratton VA Medical Center, and then stored for 1 to 2 months at 2 to 8°C before retesting at Calypte Biomedical Corp., Berkeley, Calif. Data obtained from the standard HIV urine test (4) have indicated that urine antibodies are stable for at least 55 days at room temperature and for 12 months at refrigeration temperature.

Demographic and clinical data were obtained from patient charts. Subjects were predominately males (there was one female) aged 31 to 54 years. There were 28 Caucasian, 11 black, and 4 Hispanic patients. Intravenous drug abuse or promiscuous homosexual or heterosexual behavior was noted. The AIDS surveillance case definitions of the Centers for Disease Control and Prevention (CDC) were used (5): category A, asymptomatic, progressive, generalized lymphadenopathy or acute disease; category B, symptomatic, not category A or C; and category C, AIDS indicator conditions.

Data collection. For HIV^+ patients, the results of extensive clinical laboratory blood testing, including tests for hematocrit, mean corpuscular volume, platelet count, leukocyte (WBC) count, serum protein, globulin, creatinine, glucose, amylase, serum glutamic oxalacetic transaminase, hepatitis B surface antigen, hepatitis B surface antibody, hepatitis B core antibody, hepatitis C antibody, *Toxoplasma* immunoglobulin G (IgG) and IgM, and RPR, which had been obtained in the regular course of clinical care (data not shown), were collected and recorded for analysis along with the anti-HERV antibody findings. Subsequent to the analysis of urine specimens for anti-HERV antibodies, the laboratory values for patients whose urine specimens were positive (anti-$HERV^+$) and for those that were negative (anti-$HERV^-$) for anti-HERV antibodies were compared. Laboratory analyses other than urine analyses for anti-HERV antibodies were not performed for the HIV^- control subjects. CD4 cell counts were determined by flow cytometry with standard methods. HIV loads were determined by an RNA PCR assay conducted according to procedures established by Laboratory Corporation of America.

The assay for antibody to a HERV gene product is based on an enzyme immunoassay for anti-HIV-1 antibody in urine licensed by the Food and Drug Administration (4). It is an investigational enzyme immunoassay with peptide 4.1, a synthetic 17-amino-acid peptide (H_2N-GNRLALDYLLAAEGGVC-COOH) (American Peptide Co., Sunnyvale, Calif.), the sequence of which is related to that of the envelope protein of endogenous retroviruses, as the target antigen for detecting urine antibody. For this assay, peptide 4.1 was allowed to adsorb onto each well of a microwell plate. Urine specimens and controls were then added to the wells (200 μl/well), and the plate was covered and incubated at 37°C for 60 min. Following incubation, the urine and unbound antibodies were removed by aspiration and the wells were washed six times, with 350 μl of buffered saline each time. A solution containing alkaline phosphatase conjugated to goat anti-human IgG was then added to each well (100 μl/well). The plate was then covered and incubated at 37°C for 60 min. Following incubation, the wells were washed as described above; 100 μl of a solution containing *p*-nitrophenylphosphate, the substrate for alkaline phosphatase, was added to each well; and the plate was covered and incubated at 37°C for 30 min. The enzymatic reaction was terminated by the addition of 50 μl of a stop solution containing EDTA per well. The absorbance at 405 nm was then determined spectrophotometrically. Two positive control wells and three negative control wells were included in each microwell plate. A specimen was scored positive if the absorbance was ≥0.400 and negative if the absorbance was <0.400. The cutoff value of 0.400 was established in earlier studies conducted by Calypte Biomedical Corp. on 500 presumed healthy adults (unpublished data). This value is twice the mean absorbance maximum found in this population. The specimens were randomly coded and examined in a blinded fashion, some in duplicate at the Stratton VA Medical Center and then all (singly) at Calypte Biomedical Corp. The test results for the individual specimens were highly reproducible. A subject was considered positive for anti-HERV antibody if at least one of the five urine specimens from that subject was positive.

Statistical analysis. Results were analyzed by the linear logistic model with a modified transform (9), Cochran's Q test (2), and the binomial and hypergeometric probability functions. The level of significance was 0.05.

TABLE 1. CD4 and WBC counts and viral loads of blood samples from HIV-1-positive patients

Anti-HERV antibody status	Geometric mean (range)		
	CD4 cells/mm^3	WBCs/mm^3 (10^3)	HIV-1 RNA copies/ml
Positive	85 (0–539)	4.4 (1.6–14.6)	5.1×10^4 (4×10^2–1.8×10^6)
Negative	105 (0–476)	4.3 (2.1–7.3)	3.3×10^4 (<2×10^2–4.2×10^5)

RESULTS

Demographic characteristics of the study subjects and the laboratory data were obtained during the regular course of clinical care. Demographic and laboratory findings were similar for anti-$HERV^+$ and anti-$HERV^-$ patients, except for the average serum globulin levels, which were significantly lower in anti-$HERV^+$ subjects than in anti-$HERV^-$ subjects ($P < 0.01$). Although a higher proportion of anti-$HERV^+$ (70.0%) than anti-$HERV^-$ (46.2%) subjects had liver damage (indicated by serum glutamic oxalacetic transaminase values of >28 IU/ml), the difference was not statistically significant.

Eight anti-$HERV^+$ (27%) and four anti-$HERV^-$ (31%) subjects had had no antiretroviral therapy at the start of the study. Four subjects among the group who had received >24 months of antiretroviral therapy had actually received more than 3 years of antiretroviral therapy; two anti-$HERV^+$ subjects had been treated for 46 and 59 months, and two anti-$HERV^-$ subjects had been treated for 38 and 44 months.

CD4 and WBC counts and viral load findings are summarized in Table 1. As expected, the CD4 counts and viral loads show a negative correlation ($P < 0.01$), while CD4 and WBC counts are positively correlated ($P < 0.01$). The two groups were similar with respect to CD4 count, viral load, and WBC count. In addition, for each of these parameters, the two groups were similar in variability.

Anti-peptide 4.1 antibody findings for subjects grouped according to CDC AIDS surveillance case definitions are shown in Tables 2 and 3. The proportion of anti-$HERV^+$ subjects was lower ($P < 0.01$) in CDC category A than in categories B and C (Table 2). All five urine specimens from each of the 21 control subjects were anti-$HERV^-$. For the anti-$HERV^+$ patients in each CDC category, the number of urine specimens testing positive for antibody to HERV peptide 4.1 ranged from one to five (Table 3). Home urine specimens collected at different times of day had no statistically significant differences

TABLE 2. Distribution of 43 HIV-1-positive patients by CDC clinical AIDS categories and by results of the anti-HERV antibody test[a]

CDC AIDS category	No. of patients		% Anti-HERV positive	95% CI[b]
	Anti-HERV positive	Anti-HERV negative		
A	4	7	36.4	12.6–67.3
B	13	3	81.3	64.0–92.1
C	13	3	81.3	64.0–92.1

[a] Of 21 HIV-1-negative controls, none was anti-HERV positive.
[b] CI, confidence interval.

in the proportions found to be positive for antibodies to HERV peptide 4.1 (data not shown).

DISCUSSION

Infections with exogenous retroviruses and the associated immune response to these infections have been associated with several chronic inflammatory and autoimmune diseases (18, 26, 29). Furthermore, elevated levels of HERV gene products and antibodies to these products have been associated with the same types of disease. Because of the association between antiretroviral and HERV gene product antibodies and these diseases, tests for antibodies to HERV gene products have been developed. These tests have been used to detect elevated antibody levels in the blood of animals with retroviral infections and of humans with rheumatoid arthritis, lupus erythematosus, and other diseases (3, 11, 21). However, this is the first report of the detection of elevated levels of anti-HERV antibodies in the urine of HIV-1-positive patients. The specificity of the test used in this study appears to be high, since no anti-HERV antibody was detected in the urine of any of the HIV-negative controls. However, there appears to be no correlation between the severity of HIV disease and the percentage of the five specimens from each patient that were positive. Furthermore, no information regarding the effect of HIV disease on the expression of HERV genes is available. Finally, there is currently no way to determine whether antibodies against peptide 4.1 were absent or present but undetected in the HIV-positive patients who tested negative. Consequently, the sensitivity of the test cannot be determined at this time. Nevertheless, it is interesting that only 36% of the HIV-positive patients in CDC category A were positive while 81% of those in both categories B and C were positive ($P < 0.01$). These results suggest that the test may be useful for monitoring the progression of HIV disease in patients who are initially HIV positive but negative for antibodies to the HERV-related peptide 4.1. However, to establish the utility of such a test and to address the question of whether HIV^+ and anti-$HERV^+$ patients remain anti-$HERV^+$, a longitudinal study of HIV^+ patients is required.

TABLE 3. Anti-HERV antibody findings for urine specimens of HIV^+ patients and controls

Test group	No. of patients	No. of patients with indicated no. of anti-$HERV^+$ urine specimens/total no. obtained					
		0/5	1/5	2/5	3/5	4/5	5/5
CDC AIDS category							
A	11	7	1	1	0	1	1
B	16	3	2	4	4	2	1
C	16	3	3	5	0	0	5
Control	21	21	0	0	0	0	0

In this study, there was no noteworthy association of clinical signs or symptoms with the conversion from anti-$HERV^-$ to anti-$HERV^+$ status. This implies that HIV infection alone is responsible for triggering the production of antibodies to the peptide. However, it is important to note that considerable evidence suggests that the expression of HERV genes is important in modulating the immune response and that this HERV-related immunomodulation is in some way related to the development of specific disease (10, 20, 22). Immunosuppression, as evidenced by progression to the more advanced clinical stages of HIV disease (CDC categories B and C), may be associated with diminished immunosurveillance. This could allow a higher degree of expression of HERV genes and the production of HERV gene products. In more advanced disease states associated with HIV infection, more (or more persistent) expression of HERV antigens would be expected to result in the increased detectability of antibodies to these antigens. The much lower levels of antibody potentially detectable in healthy individuals (none of the controls [$n = 21$] in this study had levels that reached the cutoff value) could reflect immune recognition of antigens occasionally produced in the immunocompetent host. Further studies may involve tests for antibodies to peptide 4.1, antibodies to other HERV peptides, and antibodies to retrovirus gene products, as well as tests for the detection of the antigens themselves. Such studies should be helpful in developing a better understanding of the association among HIV infection, the production of retrovirus-related antigens, and the production of antibodies to these antigens. In addition, investigation of the roles of cell-mediated immune modulators (cytokines) in the development of chronic inflammatory diseases could lead to a greater understanding of the initiation of anti-HERV antibody production.

In summary, the laboratory values for patients in the two HIV-positive groups in this study were similar except that there was a significantly greater prevalence of antibody to a HERV gene product detected in specimens from patients in CDC categories B and C than in those from patients in CDC category A. This suggests that progression to symptomatic stages in HIV disease correlates with the presence of antibodies to HERV gene products in urine.

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