Antigenicity of Human T-Cell Leukemia Virus-associated gp52: Greater Response in Leukemia Patients Compared to Healthy Donors Exposed to the Virus

Marjorie Robert-Guroff and Jeffrey W. Clark

ABSTRACT

Monoclonal antibody HT462 recognizes a human T-cell leukemia virus type I (HTLV-I)-associated M, 52,000 glycoprotein (HA-gp52), which is found on the surface of HTLV-infected cells. Whether HA-gp52 is encoded by the virus or by the infected cells has not yet been established. Using monoclonal HT462 in a competitive binding assay, natural human antibodies specific for HA-gp52 were detected in 97% of the patients from the United States, the Caribbean, and Japan with adult T-cell leukemia but not in healthy donors not exposed to HTLV-I. In contrast, antibodies to HA-gp52 occur in healthy virus-exposed donors, but at a lower prevalence than that observed in patients. Among Japanese from HTLV-I-endemic areas and exposed to the virus as indicated by the presence of antibodies to disrupted HTLV-I, 93% of adult T-cell leukemia patients who were seropositive for HA-gp52 compared to only 16% of healthy individuals. Differing sensitivities in the methods of assaying antibodies to HTLV and HA-gp52 were not responsible for these observations as shown by the lack of correlation of HTLV-I antibody titer with the presence of antibody to HA-gp52. Among adult T-cell leukemia patients, antibody titers to HTLV-I and HA-gp52 also varied independently. These results indicate that HA-gp52 in humans is antigenic and correlated with disease. Detection of antibody to this protein in asymptomatic individuals may be indicative of a predisease condition.

INTRODUCTION

The family of human T-cell leukemia (lymphotropic) viruses, designated HTLV, comprises a group of related, exogenous retroviruses which exert either profound proliferative or cytopathic effects on the target T-lymphocytes they infect. Three distinct subtypes have been identified. The prototype HTLV-I appears to be the etiological agent of mature T-cell leukemias and lymphomas typified by ATL (1). HTLV-II, isolated from cells of a patient with T-cell hairy cell leukemia (2), also exerts lymphoproliferative effects; however, to date, it has not been associated with a particular human cancer. The most recently described family member, HTLV-III (3, 4), is strongly cytopathic to infected T-cells and is most likely the etiological agent of the acquired immunodeficiency syndrome. Questions crucial to understanding the interaction of the HTLV family members with T-cells concern the identification and elucidation of the mechanism of action of the viral sequences and their encoded protein products, which are directly or indirectly responsible for both the transforming and killing ability of the viruses.

We have recently described a monoclonal antibody, termed HT462, which is specific for an M, 52,000 glycoprotein associated with HTLV-I (HA-gp52) and found on the surface of HTLV-I-infected cells (5). The viral association of this glycoprotein is supported by its concomitant expression with viral structural proteins upon initiation of viral synthesis in HTLV-infected cells, as well as its coincident banding with HTLV in density gradients of metrizamide. HA-gp52 is found only on HTLV-infected cells, including those producing as well as those not producing virus proteins. The protein moiety of this molecule has a molecular weight of approximately 42,000. HA-gp52 is clearly not a product of the HTLV gag gene which encodes the mature viral core proteins p19, p24, and p15 (6–9). Nor is it a product of the poly gene which encodes the viral reverse transcriptase of approximately 100,000 molecular weight (10). Whether it is encoded by a portion of the env-pX genetic region (11), whether it arises as a variant cleavage product of a viral precursor protein or by a splicing mechanism, or whether it is a celluly encoded protein induced following viral infection has not yet been established. Before pursuing further its nature, it was important to know if HA-gp52 was relevant in the known association of the virus with disease. We therefore analyzed sera of ATL patients and healthy individuals for natural antibodies to the HA-gp52 using monoclonal antibody HT462 in a competitive binding assay. Our results suggest that HTLV-I-positive patients are more exposed to HA-gp52 than are HTLV-I-positive healthy donors as indicated by an increased antibody prevalence in the former group. Whether or not HA-gp52 is involved in the mechanism of T-cell transformation, serological detection of antibody to this protein may be a marker of disease and may signal early pathogenesis.

MATERIALS AND METHODS

Viral Antigens. The purification of HTLV-I from the HUT 102 producer cell line by banding in sucrose density gradients has been previously described (12). The procedure for disrupting HTLV and preparing a viral lysate has also been published (13). This viral lysate was used as the test antigen in the procedures described below.

Antibodies. The preparation and characterization of monoclonal antibody HT462 have been recently described (5). Briefly, BALB/c mice were immunized with preparations of disrupted HTLV-I. Immune spleen cells were fused, using polyethylene glycol with the nonsecreting mouse myeloma line, NS-1. Hybridomas producing antibodies to HTLV-I antigens were selected first by ELISA test and subsequently by indirect membrane immune fluorescence for reactivity with HTLV-producing cell lines. Antibody produced by hybridoma line HT462 was reactive in both systems, was characterized extensively, and was inoculated into BALB/c mice in order to obtain monoclonal antibody-rich ascites fluids. The IgG1 produced by the HT462 line was purified from ascites fluid on
Antibody to HTLV-associated gp52

Protein A: Sepharose (14). Following dialysis and concentration by negative pressure, the HT462 IgG was iodinated using chloramine T (15).

Other monoclonal antibodies used as competitors included monoclonal anti-HTLV p19 from the cell line designated 12/1-2 (16) and anti-HTLV p15 from the HT5 cell line. Ascites fluids derived from inoculation of the P3x63 IgG-producing mouse myeloma cell line into BALB/c mice were used as control.

Antisera produced in rabbits or goats by inoculation of purified antigen or disrupted virus preparations and used here as controls included monospecific rabbit and goat sera to HTLV p24, monospecific rabbit sera to both HTLV p15 and p19, and polyspecific rabbit and goat sera reactive with antigens of disrupted HTLV (8).

Human sera were obtained from infected or lymphopenic donors. Following reconstitution if necessary, they were stored at −70°C. The majority of serum samples tested had been previously thawed for assay of antibodies to disrupted HTLV-I.

Immunoassays. A competitive binding assay based on the ability of human sera to compete with a monoclonal antibody for binding to an HTLV antigen was previously used to illustrate the presence of antibodies in human serum reactive with HTLV p19 (17). A similar assay was carried out here. HTLV-I (5 μg/ml) in 100-μl aliquots of carbonate-bicarbonate buffer, pH 9.6, was incubated overnight in "Removewells" of an Immulon microtiter plate (Dynatech) in order to bind viral antigens to the plastic. The plate was rinsed with water, and a constant amount of 125I-HT462 IgG, giving approximately 50% saturation of available antigen binding sites, was mixed with serial dilutions of test human sera in phosphate-buffered saline containing 10% fetal calf serum, 10% normal goat serum, and Trasylol (FBA Pharmaceuticals, New York, NY) at a final dilution of 1:50. Plates were incubated overnight at 4°C. Following washes with phosphate-buffered saline containing 0.05% Tween 20, the wells of the plates were counted individually in a Searle gamma counter. The cpm of 125I-HT462 IgG bound to the HTLV-coated wells was normalized to the cpm bound in the absence of human serum.

Results were expressed as the percentage of control consisting of cpm bound in the presence of serial dilutions of a standard negative human serum. Titer was expressed as the reciprocal of the serum dilution at which the test serum bound 75% of the control value. Antisera, prepared in rabbits or goats, or mouse monoclonal antibodies were tested in the same way, except the appropriate rabbit or goat normal serum or a mouse ascites containing IgG was used as a control.

Antibodies to HTLV-I antigens present in preparations of disrupted virus were assayed by the ELSIA technique (18). Confirmation of viral specificity of observed antibody reactivities was determined by competition assays as previously described (19). Positive sera were titered in the ELSIA system. Titer is expressed as the reciprocal of the serum dilution at which the absorbance obtained using the test serum equals the absorbance obtained with a standard negative serum diluted 1:20.

RESULTS

Specificity of Competitive Binding Assay for Natural Human Antibodies to HA-gp52. The specificity of the competitive binding assay for antibody to HA-gp52 was assessed using a variety of monoclonal antibodies and polyclonal animal sera. Chart 1 illustrates typical results. Both rabbit and goat sera prepared against whole disrupted HTLV competed well in the assay for binding to the HT462 antigen (Chart 1A). However, rabbit and goat sera prepared against purified HTLV gag proteins p19, p24, and p15, including a goat anti-p24 serum with extremely high titer, failed to compete significantly. Similarly, mouse monoclonal antibodies to HTLV p19 and p15 did not compete in the system, while unlabelled HT462 IgG completely abolished the binding of the iodinated homologous IgG in the assay (Chart 1B). Human sera when used as competitors in the assay either competed or failed to compete (Chart 1C), clearly indicating that some human sera possess antibodies to HA-gp52.

Prevalence of Natural Human Antibodies to HA-gp52. A preliminary survey of sera from ATL patients and healthy individuals showed that 90% (37 of 41) of ATL cases, including patients from Japan, the Caribbean, and the United States, possessed antibody to the HT462 antigen. In contrast, none of the random healthy donors from the United States tested (0 of 17) possessed this antibody. However, antibody to HA-gp52 was detected in sera of healthy donors when either populations endemic for HTLV-I or relatives of ATL patients were analyzed. We therefore asked whether antibody to HA-gp52 was simply correlated with the presence of antibody to other HTLV-I antigens. Table 1 summarizes the results. Among HTLV antibody-positive ATL patients, 97% also possessed antibody to HA-gp52. In contrast,

"M. Robert-Guroff, unpublished data.

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only 22% of the HTLV antibody-positive healthy donors were also positive for HA-gp52-specific antibody. The healthy donors from Japan who were analyzed were all random donors, while the individuals tested from the United States and the Caribbean included both random donors and relatives of ATL patients. While the percentage of anti-HA-gp52-positive sera in the latter samples was higher compared to the overall percentage among healthy donors, the possible biological significance of this difference was not pursued as the number of samples involved was small. We did, however, further examine the Japanese healthy donor sera, where only 16% of HTLV antibody-positive sera also possessed anti-HA-gp52 reactivity.

Chart 2 indicates that the significantly lower prevalence of antibody to HA-gp52 among HTLV-I-seropositive Japanese random healthy donors compared to Japanese ATL patients is not correlated with overall antibody titer to HTLV-I antigens. Many sera with extremely high titers to disrupted HTLV lacked reactivity to the HA-gp52. The reverse was also true, that some sera with low titer to HTLV-I antigens possessed antibody to the HA-gp52. Thus, the lower anti-HA-gp52 prevalence seen among healthy Japanese donors in contrast to that observed in ATL patients was not a result of lesser sensitivity of the competitive binding assay compared to that of the ELISA technique used to detect antibodies to disrupted HTLV-I preparations.

Similarly, analysis of results obtained with sera of ATL patients showed no dependence on assay sensitivity. Titers of sera positive for antibodies to HA-gp52 were not correlated with titers to other HTLV-I antigens (Chart 3). While we observed a tendency for anti-HA-gp52 titers to be elevated in more advanced stages of disease compared to those seen in either healthy normals or in patients with less advanced disease (data not shown), the numbers of samples in each category were too small to make an adequate conclusion on this point.

**DISCUSSION**

The studies reported here show that the HA-gp52 recognized by monoclonal antibody HT462 is antigenic in humans and associated with ATL. Hence, it is relevant as a "tumor-specific" and possibly also as a viral-specific antigen. Antibody to HA-gp52 was not detected in random healthy donors from non-HTLV-endemic areas, yet antibody prevalence was nearly 100% among all ATL patients tested. Thus, the antigen must be routinely expressed in the individuals with disease. Furthermore, the observation that, among known HTLV antibody-positive donors, both healthy and diseased, the ATL patients possess antibody to HA-gp52, yet the healthy donors have a much lower antibody prevalence, indicates that the antigen is more highly expressed in the patient population. Based on these results, one may speculate that the association of HA-gp52 with ATL may provide an important marker of disease development. The possibility also exists that the antigen is important mechanistically in the progression from HTLV infection to manifestation of ATL. Resolution of these points will require both long-term studies of seropositive individuals and serological analysis of patients in various stages of disease.

The nature of the antigen recognized by monoclonal antibody HT462 is not yet clear. Based on the size of the antigen, its glycosylation, its location on the surface of infected cells, and its association with the virus on a density gradient as well as during synthesis of viral proteins, it seemed a likely candidate for the viral envelope glycoprotein. While HTLV-II has been described (20) as possessing an envelope protein with a molecular weight of 52,000, the envelope glycoprotein of HTLV-I has recently been described as an M, 46,000 glycoprotein with a precursor of approximately 61,000 to 67,000 molecular weight (7, 20–23). Although the antigen we detect might be a variant cleavage product of the envelope precursor, the fact that the antigen is detected by indirect immune fluorescence assays on the surface of HTLV-infected cells which are not producing viral proteins (5), as well as on an HTLV-infected cell line lacking proviral sequences of the envelope gene (11) indicates that the antigen is more highly expressed in the patient population. Based on these results, one may speculate that the association of HA-gp52 with ATL may provide an important marker of disease development. The possibility also exists that the antigen is important mechanistically in the progression from HTLV infection to manifestation of ATL. Resolution of these points will require both long-term studies of seropositive individuals and serological analysis of patients in various stages of disease.
typical of the HTLV-I-producing HUT102 cells, or a viral induced but cellular coded antigen. These possibilities will be resolved following the isolation of the HA-gp52 and sufficient analysis of its sequence in order to determine if it is viral encoded. In addition, the analysis of the proteins of various HTLV-infected cell lines detected by the monoclonal antibody will further elucidate the nature of the HA-gp52. These studies are under way.

Regardless of the nature of HA-gp52, our data suggest that it may be linked with development of disease. Isolation of the protein will allow investigation of possible biological activity. The monoclonal antibody HT642, specific for HA-gp52, now allows broader detection of HTLV-infected cells by specifically recognizing both HTLV-producer cells and HTLV-infected nonproducer cells (5) and may also be used in serological assays to possibly presage impending disease states.

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REFERENCES

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