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Human T-cell lymphotropic virus (HTLV)-related endogenous sequence, HRES-1, encodes a 28-kDa protein: A possible autoantigen for HTLV-I gag-reactive autoantibodies

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ABSTRACT **The presence of a human T-cell lymphotropic virus (HTLV)-related endogenous sequence, HRES-1, in the human genome has been documented. The HRES-1 genomic locus is transcriptionally active and contains open reading frames. Antibodies 232 and 233, specific for synthetic peptides pep14–24 and pep117–127, corresponding to two nonoverlapping HTLV-related regions in the longer open reading frame of HRES-1, recognize an identical 28-kDa protein in H9 human T cells. Thus, HRES-1 is a human endogenous retroviral sequence capable of protein expression. HRES-1/p28 is localized to the cytoplasm and nuclear bodies. While HTLV-I-specific antibodies react with HRES-1 peptides, antibody 233 cross-reacts with HTLV-I gag p24 protein. Three consecutive highly charged amino acid residues, Arg-Arg-Glu, present in both HRES-1 pep117–127 and HTLV-I gag p24 are likely to be the core of cross-reactive epitopes. The prevalence of antibodies to HRES-1 peptides pep14–24 and pep117–127 was determined in 65 normal blood donors and 146 patients with immunological disorders. Sera of patients with multiple sclerosis (19 out of 65, 29%), progressive systemic sclerosis (4 out of 17, 23%), systemic lupus erythematosus (4 out of 19, 21%), and Sjogren syndrome (2 out of 19, 10%) contained significantly higher HRES-1 peptide binding activity than sera of normal donors. Sera of patients with AIDS showed no specific binding to HRES-1 peptides. Nine of 30 HRES-1-seropositive patients showed immunoreactivity to HTLV-I gag p24. The data indicate that HRES-1/p28 may serve as an autoantigen eliciting autoantibodies cross-reactive with HTLV-I gag antigens.**

The possibility of a retroviral etiology has long been raised for a number of immunological disorders (1, 2). Several autoantigens such as U1 small nuclear ribonucleoprotein (3), topoisomerase I (4), and SS-B/La (5) share cross-reactive epitopes with murine retroviral gag proteins. None of these autoantigens show amino acid sequence homology with known human retroviruses. Antibodies reacting with gag proteins of human T-cell lymphotropic virus I (HTLV-I) were described in patients with systemic lupus erythematosus (SLE) (6) and multiple sclerosis (MS) (7–9). However, the presence of HTLV-I, HTLV-II, or related exogenous retroviruses could not be conclusively associated with these diseases (10–13). This raised the possibility that the natural targets of HTLV-I-reactive antibodies of patients with autoimmune disease may correspond to endogenous retroviral sequences (ERSs).

ERSs are considered a major factor in shaping and reorganization of the eukaryotic genome (14). Whereas exogenous retroviruses are infectious, with a replication cycle that requires integration of proviral DNA into host cell DNA, ERSs are transmitted genetically in a classical Mendelian fashion through the germ line as proviral DNA. The normal human genome contains a complex variety of ERSs. These human ERSs were isolated by low-stringency hybridization to known mammalian ERSs (15–18), by hybridization to the 3′ terminus of tRNAs (19, 20), or during analyses of flanking regions of other genes (21, 22). To date, none of these ERSs has been shown to encode a protein, and they have not been implicated in human disease.

We have earlier documented the presence of a human T-cell lymphotropic virus type I (HTLV-I)-related endogenous sequence, termed HRES-1 (GenBank accession no. X16514), in the human genome (23). The HRES-1 locus is transcriptionally active and contains open reading frames (ORFs). Flanking region 5′ to the ORFs contains a TATA box, a polyadenylylation signal, a potential tRNA primer binding site, and characteristic inverted repeat sequences at locations that are typical of a retroviral long terminal repeat (23). Hybridization analysis with genomic DNA samples of selected phylogenetic stages revealed that HRES-1 is apparently confined to the primate lineage. The data suggest that the HRES-1 sequence was not phylogenetically inherited but entered the genome of Old World monkeys as an exogenous element, and the human genomic HRES-1 sequence might have originated from an as yet unidentified exogenous retrovirus. HRES-1 is represented as a single-copy element per haploid genome, which has now been mapped to a common fragile site of chromosome 1 at 1q42 (24). The present data suggest that HRES-1 is capable of protein expression. HRES-1 protein contains antigenic epitopes cross-reactive with gag of HTLV-I. Antibodies to HRES-1-specific synthetic peptides were noted in patients with MS, progressive systemic sclerosis (PSS), SLE, Sjogren syndrome (SJS), and essential cryoglobulinemia (ECG). The data suggest that HRES-1 may serve as an autoantigen and correspond to a natural target of HTLV-I core protein-reactive autoantibodies.

MATERIALS AND METHODS

Peptides. HRES-1-specific 11-amino-acid-long peptides, pep14–24 (PTRAPSGPRPP) and pep117–127 (RRREGPDRSPR) were synthesized based on antigenicity and hydrophobicity analysis as well as relatedness to HTLV-I (23). Amino acid residues homologous to gag regions of HTLV-I

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Abbreviations: HTLV, human T-cell lymphotropic virus; HRES, HTLV-related endogenous sequence; ERS, endogenous retroviral sequence; PSS, progressive systemic sclerosis; MS, multiple sclerosis; SLE, systemic lupus erythematosus; SJS, Sjogren syndrome; ECG, essential cryoglobulinemia; ORF, open reading frame; Ab, antibody.

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are underlined. Designation of amino acid residues corresponds to the translated amino acid sequence of the p25 ORF of HRES-1 (23). Amino acid residues 14–24 correspond to a region of HRES-1 with homology to gag p19, whereas amino acid residues 117–127 correspond to a region of HRES-1 with homology to gag p24 of HTLV-I/HTLV-II (23). After peptide synthesis, authenticity of amino acid sequences was ensured by HPLC analysis (Multiple Peptide Systems, San Diego). Twenty milligrams of each peptide was conjugated with keyhole limpet hemocyanin carrier protein.

Sera. As a source of antibodies, sera of 146 patients with immunological disorders were utilized. Patients included 19 with SJS, 17 with PSS, 19 with SLE, 18 with ECG, 65 with MS, and 8 with AIDS. All patients satisfied the criteria for a definitive diagnosis (25). Sera from 65 normal blood donors were used as negative controls. As HTLV-I-specific antibodies, sera of 5 adult T-cell leukemia patients and a rabbit antibody raised against HTLV-I virion lysate were utilized. HRES-1 peptide-specific rabbit antisera 232 and 233 were generated by immunization with keyhole limpet hemocyanin-conjugated peptides pep14–24 and pep117–127, respectively. Antibody (Ab) 1260 (HTLV-I gag p19-, p29-, p33-, and p53-specific monoclonal antibody, which does not react with native or recombinant gag p24) was obtained from Genzyme.

Preparation of HTLV-I Antigens. HTLV-I-infected SLB-I cells (kindly provided by Irvin Chen, University of California, Los Angeles) were centrifuged at 1000 × *g* for 10 min at 4°C. Supernatants were filtered through a 0.2-μm filter, and virions were purified by ultracentrifugation in a 20–50% continuous sucrose gradient (26,000 × *g*, 4 hr, 4°C). Virions were lysed in SDS/PAGE sample buffer, separated in a 12% polyacrylamide gel, and electroblotted to nitrocellulose (25). The virion lysate was essentially free of cellular proteins as controlled by the absence of HRES-1/p28 and lack of detectability of a highly abundant cytoplasmic protein, HRES-2. HTLV-I gag p24 lacking its N-terminal 14 amino acids was expressed in *Escherichia coli*. Recombinant protein was gel purified, reelectrophoresed in SDS/PAGE, and electroblotted to nitrocellulose (26). Antibodies to HTLV-I proteins were studied by Western blot analysis. As HTLV-I negative cells, the H9 human leukemic T-cell line was utilized. H9/1 and H9/2 are subclones of H9 cells originating from Robert Gallo's laboratory (National Institutes of Health).

Western Blot Analysis. Protein lysates from 2×10^5 cells per well were separated by SDS/PAGE and electroblotted to nitrocellulose (26). For testing of human sera, nitrocellulose strips were incubated in 100 mM Tris, pH 7.5/0.9% NaCl/0.1% Tween 20/5% skim milk, with serum samples at a 100-fold dilution overnight at room temperature. After washing, the strips were incubated with biotinylated goat anti-human serum and, subsequently, with horseradish peroxidase-conjugated avidin (The Jackson Laboratory). For primary rabbit antibodies, after washing, the blots were further incubated with horseradish peroxidase-conjugated goat anti-rabbit IgG. In between incubations, the strips were vigorously washed in 0.1% Tween 20/100 mM Tris, pH 7.5/0.9% NaCl. For the primary mouse antibody, after washing, the blot was further incubated with biotinylated goat anti-mouse IgG and subsequently with horseradish peroxidase-conjugated avidin. Blots were developed with a substrate comprised of 4-chloronaphthol at 1 mg/ml and 0.003% hydrogen peroxide.

Confocal Laser Scanning Microscopy. Cells were permeabilized by treatment with 90% methanol in phosphate-buffered saline (PBS) for 15 min at −20°C. Subsequently, all incubations and washing were done in PBS containing 0.002% Triton X-100. Cells were incubated at 37°C with rabbit antibodies at a 1:100 dilution. After washing, cells were further incubated with fluorescein isothiocyanate-conjugated goat anti-rabbit IgG (Tago). Specimens were examined with a Nikon Optiphot microscope interfaced with a Bio-Rad MRC-600 confocal laser scanning microscope having the following configuration: 25-mW argon laser, 488 and 514 maximum lines, 80386 35-MHz PC/AT computer with an AGA optical disk drive, 650 megabyte 3M rewritable optical disk cartridges, 768 × 512 pixel image storage, control software for image acquisition of *x-y* scan, *z*-series scan, three-dimensional visualization, and automated time-lapse recording. Photographs were taken on Kodak TMax ASA 100 film.

ELISA. Ninety-six-well plates were precoated at 4°C overnight with 1 μg of peptide per well in 0.01 M $NaHCO_3$ (pH 9.55). Uncoated sites of the wells were blocked with 10% goat serum/0.1% Tween 20 in PBS at pH 7.4 at room temperature for 1 hr. Human sera were diluted 1:50 with 10% goat serum/0.1% Tween 20 in PBS and added to the wells in triplicate. Rabbit antibodies were applied in the same solution at a dilution of 1:300. After incubation for 1 hr, the plates were washed six times with 0.1% Tween 20 in PBS. The plates were incubated further with horseradish peroxidase-conjugated goat anti-human and anti-rabbit antibodies, respectively, at room temperature for 1 hr. The plates were washed six times and developed with 2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonic acid). Absorbance values were read at 405 nm.

Statistics. ELISA values indicate the mean ± SD of absorbance data at 405 nm from triplicate wells. Immunoreactivities to HRES-1 peptides for individual donor groups are given as the mean ± SEM. Statistical analysis was performed with Student's *t* test.

RESULTS

Two HRES-1-specific 11-amino-acid-long peptides were synthesized. They correspond to the two nonoverlapping HTLV-related regions of the HRES-1 p25 ORF, residues 14–24 (pep14–24) and residues 117–127 (pep117–127) (23). Rabbit antisera, Ab 232 and Ab 233, were raised against the two synthetic peptides. Abs 232 and 233 were found to be highly specific for their respective peptide antigens (Table 1). By Western blot analysis both antibodies recognize a protein of identical molecular mass in the H9 human T-cell leukemia cell line (Fig. 1). The size of this protein is ≈28 kDa. This is in agreement with the calculated molecular weight of the HRES-1 protein, 28,341.36, based on its amino acid composition. HRES-1/p25 was formerly designated based on the length of the ORF in the DNA sequence (23). To our knowledge, HRES-1 is the first human ERS shown to be capable of protein expression.

Expression of HRES-1/p28 appears to be cell type-specific (Fig. 2). High-level HRES-1 expression was noted in H9 T cells, whereas no significant staining of DG75 Burkitt lymphoma B cells was noted (Fig. 2*C*). Expression of HRES-1/p28 is localized to the cytoplasm and to nuclear bodies in H9 cells. Localization of staining to intranuclear bodies was confirmed by computerized imaging at various intracellular depths (Fig. 2 *A* and *B*). As a control, H9 cells were stained with Ab 169, which is specific for an intracytoplasmic protein

Table 1. Specificity analysis by ELISA of rabbit sera 232 and 233 raised against HRES-1 peptides pep14–24 and pep117–127, respectively

Antigen	Primary antibody	A_{405}
pep14–24	Preimmune 232	0.354
pep14–24	232	2.775
pep14–24	233	0.226
pep117–127	Preimmune 233	0.309
pep117–127	232	0.347
pep117–127	233	2.744

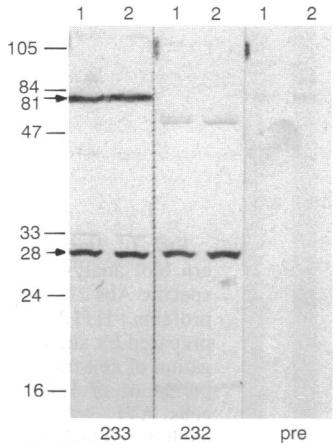

FIG. 1. Western blot analysis of protein lysates of H9/1 (lanes 1) and H9/2 (lanes 2) cells. Both Ab 232, specific for HRES-1 peptide pep14–24, and Ab 233, specific for HRES-1 peptide pep117–127, recognize a common 28-kDa protein. pre, preimmune rabbit serum. An 81-kDa protein was recognized only by antibody 233. Size markers (in kDa) are indicated at left.

encoded by another human ERS, HRES-2 (K.B. and A.P., unpublished data) (Fig. 2*D*).

To determine if HRES-1 peptides contain antigenic epitopes cross-reactive with HTLV-I proteins, the reactivity of HTLV-I antibodies to HRES-1 peptides was evaluated. As shown in Table 2, anti-HTLV-I rabbit antibody and serum of an HTLV-I-infected adult T-cell leukemia patient react with both HRES-1 peptides. On the other hand, Ab 233, specific for the HTLV-I gag p24-related region of HRES-1, showed strong reactivity with HTLV-I gag p24 and the 53-kDa gag precursor protein (Fig. 3). Ab 232 showed reactivity to the p53, whereas no binding to HTLV-I gag p19 was noted (Fig. 3). Three consecutive highly charged amino acid residues, Arg-Arg-Glu, present in both HRES-1 pep117–127 and HTLV-I gag p24 (23), are likely to be responsible for the cross-reactivity. Sera of 11 patients with immunological disorders including 9 with MS, 1 with ECG, and 1 with SLE showed immunoreactivity to recombinant HTLV-I gag p24 protein (data not shown). Prototype HTLV-I antibody activity against HTLV-I virion lysates by Western blot analysis and HTLV-I DNA were absent in these patients (17). Nine of these patients demonstrated specific binding to both of the HRES-1 peptides (Table 2). The data indicate that the natural target of HTLV-I-reactive antibodies in sera of patients with immunological disorders may correspond to HRES-1, a self-antigen harboring epitopes cross-reactive with HTLV-I gag protein.

The prevalence of antibodies to HRES-1 peptides pep14–24 and pep117–127 was studied in sera of 65 normal blood donors and 146 patients with immunological disorders. A significant correlation of anti-pep14–24 and anti-pep117–127 activities was observed throughout the donor population examined—that is, donors with high pep14–24 binding also showed high pep117–127 binding and donors with low pep14–24 binding showed low pep117–127 binding as well. No correlation was found between HRES-1 peptide binding and immunoglobulin concentrations in the sera of 7 patients and 4 control donors (data not shown). Sera of groups of patients with MS, PSS, SLE, and SJS contained significantly higher HRES-1 peptide binding activity than sera of normal donors (Table 3). Sera of patients with ECG and AIDS showed no higher binding activities to HRES-1 peptides than sera of normal donors (Table 3). A cut-off level used to determine individual seropositivity was calculated as the mean plus two standard deviations of normals. The cut-off level in the ELISA was 0.599 for pep14–24 and 0.551 for pep117–127. Donors were considered HRES-1 seropositive if the sera contained antibody activity against both of the HRES-1 peptides. Thus, 29% of patients with MS, 23% of patients with PSS, 21% of patients with SLE, 10% of patients with SJS, 5% of patients with ECG, none of the patients with AIDS, and 1.5% of normal donors were considered HRES-1 seropositive (Table 4).

DISCUSSION

Unlike murine and baboon endogenous proviruses, human ERSs were not known to produce infectious retroviral particles or even retroviral proteins. Transcription of long terminal repeat-like and env-like regions of a full-length human ERS, 4-1, was detected in human placenta, breast, and colon carcinoma cells (27). Since sequences of cDNA clones corresponding to the mRNA species revealed in-frame termination codons, 4-1 could not encode env proteins. In contrast, HRES-1, which is transcribed in various human tissues and cell lines, does contain ORFs. As described earlier, within the deduced amino acid sequence of HRES-1/p25, residues 6–36 and residues 104–139 show a significant homology to

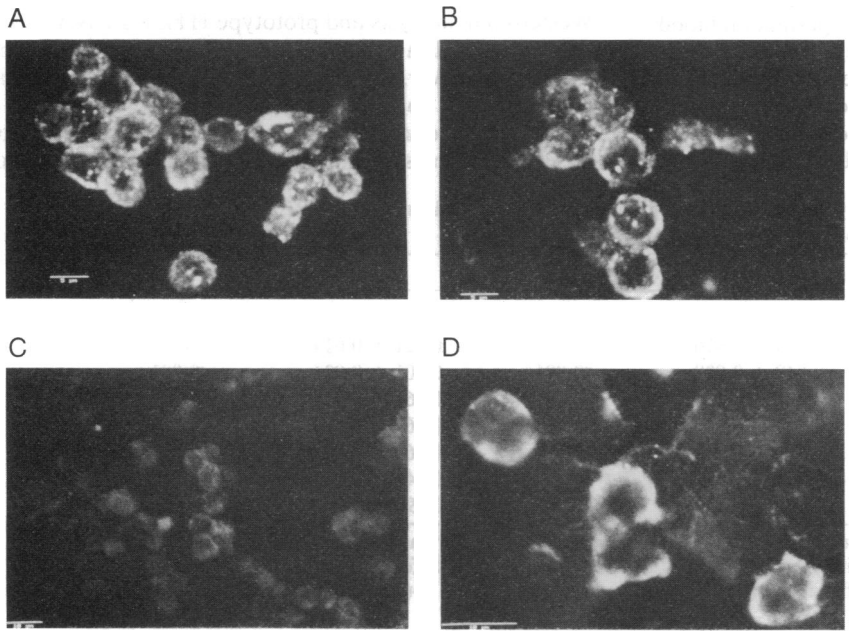

FIG. 2. Immunofluorescence staining of nuclear bodies in H9 cells with HRES-1-specific Ab 232 at intracellular depths of 18 μm (*A*) and 21 μm (*B*). (*C*) Staining of DG75 cells with Ab 232. (*D*) Staining of H9 cells with Ab 169, specific for the intracytoplasmic protein HRES-2.

Table 2. Immunoreactivity of sera reactive with recombinant HTLV-I gag p24 protein with HRES-1 peptides pep14–24 and pep117–127

Antibody	A_{405}	
	pep14–24	pep117–127
232	2.060 ± 0.220	0.247 ± 0.005
233	0.181 ± 0.021	2.134 ± 0.040
Anti-HTLV-I rabbit	1.108 ± 0.022	0.675 ± 0.033
Normal human serum no. 1	0.230 ± 0.002	0.228 ± 0.008
Normal human serum no. 2	0.338 ± 0.025	0.296 ± 0.028
HTLV-I/human*	0.608 ± 0.077	0.590 ± 0.055
BEN (ECG)*	0.758 ± 0.006	0.764 ± 0.024
SZA (MS)*	0.621 ± 0.062	0.564 ± 0.065
ALL (MS)*	0.588 ± 0.040	0.536 ± 0.065
VAS (MS)*	0.826 ± 0.065	0.707 ± 0.010
FUG (MS)*	0.646 ± 0.016	0.527 ± 0.021
ROB (MS)*	0.891 ± 0.017	0.862 ± 0.015
FER (MS)*	0.605 ± 0.037	0.814 ± 0.007
BUR (MS)*	0.629 ± 0.057	0.565 ± 0.043
PUS (SLE)*	0.820 ± 0.026	0.923 ± 0.017

Data represent the mean ± SEM from triplicate wells.
*Recombinant HTLV-I gag p24 binding antibody.

gag regions of HTLV-I, HTLV-II, human immunodeficiency virus type 2, and feline sarcoma virus (23). Homologies in HRES-1 residues 6–36 and 104–139 correspond to HTLV-I gag p19 and gag p24, respectively (23, 28). The longer ORF of HRES-1 was originally designated as p25 based on the number of nucleotides in the sequence. The calculated molecular weight of the translated amino acid sequence of p25 ORF is 28,341. Thus, the protein encoded by the longer ORF has been redefined as a 28-kDa protein (HRES-1/p28). By Western blot analysis, rabbit Abs 232 and 233, which are highly specific for two nonoverlapping HRES-1 peptides, recognize a protein of identical molecular mass. The size of this protein is ≈28 kDa. Thus, to our knowledge, HRES-1 is the first human ERS shown to be capable of protein expression. Ab 233, in addition to recognizing HRES-1/p28, shows immunoreactivity to another 81-kDa protein species. Identity of this protein and its relationship to HRES-1/p28 remain to be determined.

Expression of HRES-1/p28 appears to be cell type specific. A high level of HRES-1 expression was noted in H9 cells, whereas no significant staining of DG75 Burkitt lymphoma cells was noted. HRES-1/p28 was demonstrated in several lymphoblastoid T-cell lines, normal peripheral blood T cells, and in normal brain and liver by Western blot analysis and in cutaneous T-cell lymphoma tissues by immunohistochemistry using the 28-kDa band-specific Ab 232. Expression of HRES-1/p28 is localized to the cytoplasm and to nuclear bodies, possibly nucleoli, in H9 cells. On the basis of confocal immunofluorescence microscopy and flow cytometry, HRES-1/p28 is not expressed on the cell surface. Intracellular location renders HRES-1 relatively unexposed to the immune system, which is characteristic of human autoantigens (25).

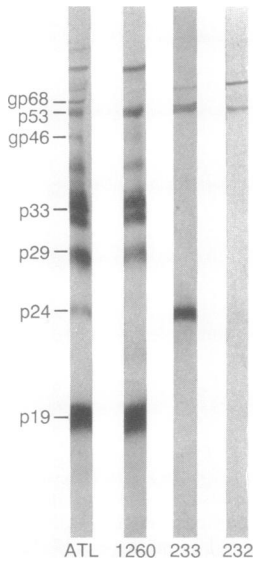

FIG. 3. Immunoreactivity by Western blot analysis of HRES-1 peptide-specific Abs 232 and 233 with HTLV-I proteins. HTLV-I virion lysate was prepared by sucrose gradient centrifugation of cell-free (0.2-μm-filtered) supernatant of HTLV-I-infected SLB-I cells. ATL, serum of HTLV-I-infected adult T-cell leukemia patient. Ab 1260, HTLV-I gag p19-, p29-, p33-, and p53-specific murine monoclonal Ab, which does not react with native or recombinant gag p24.

Reactivity of HTLV-I antibodies to HRES-1 peptides confirmed that HRES-1 and HTLV-I contain cross-reactive antigenic epitopes. Ab 233, specific for HRES-1 peptide pep117–127, showed strong reactivity with HTLV-I gag p24. Ab 232 showed reactivity to the p53, whereas no binding to HTLV-I gag p19 was noted (Fig. 3). This suggests that HRES-1 and HTLV-I gag p19 may not contain strong cross-reactive epitopes or that peptides longer than pep14–24 or a recombinant HRES-1 protein may be needed to raise an antiserum of higher affinity to HTLV-I gag p19. Three consecutive highly charged amino acid residues, Arg-Arg-Glu, present in both HRES-1 pep117–127 and HTLV-I gag p24 (23) are likely to be responsible for the cross-reactivity. Sera of 11 patients with immunological disorders including 9 with MS, 1 with ECG, and 1 with SLE showed immunoreactivity to recombinant HTLV-I gag p24 protein (data not shown). This is in accordance with earlier data showing similar frequency of HTLV-I gag p24-reactive antibodies in patients with MS (7–9). Antibodies to all characteristic HTLV-I virion proteins, using Western blot analysis and prototype HTLV-I DNA based on polymerase chain reaction, were absent in these patients (10–13). However, 9 of the HTLV-I gag p24-reactive patients had antibodies to both of the HRES-1 peptides. The data indicate that the natural target of HTLV-I-reactive antibodies in sera of patients with immunological disorders may corre-

Table 3. Immunoreactivity of sera from patients with immune disorders against HRES-1-specific peptides pep14–24 and pep117–127

Donors (*n*)	pep14–24		pep117–127	
	A_{405}	*P*	A_{405}	*P*
Normal donors (65)	0.333 ± 0.016	—	0.321 ± 0.014	—
MS patients (65)	0.549 ± 0.028	<0.001	0.501 ± 0.024	<0.001
PSS patients (17)	0.502 ± 0.055	<0.001	0.461 ± 0.048	<0.001
SLE patients (19)	0.488 ± 0.048	<0.001	0.462 ± 0.045	<0.001
SJS patients (19)	0.436 ± 0.041	<0.01	0.469 ± 0.043	<0.001
ECG patients (18)	0.371 ± 0.036	NS	0.343 ± 0.037	NS
AIDS patients (8)	0.285 ± 0.031	NS	0.299 ± 0.038	NS

Data represent the mean ± SEM of ELISA absorbance values for each group tested. Statistical analysis was performed with Student's *t* test. *P* values indicate the difference in HRES-1 peptide-specific immunoreactivity for each group of patients in comparison to normal blood donors. NS, not significant.

Table 4. Frequency of HRES-1 seropositivity in patients with immunological disorders

Diagnosis	Total	Seropositive, no.	Frequency, %
MS	65	19	29
PSS	17	4	23
SLE	19	4	21
SJS	19	2	10
ECG	18	1	5
AIDS	8	0	0
Controls	65	1	1.5

The cut-off level in the ELISA was 0.599 for pep14–24 and 0.551 for pep117–127. Donors were considered HRES-1 seropositive if the sera contained antibody activity against both of the HRES-1 peptides.

spond to HRES-1, a self-antigen harboring epitopes cross-reactive with HTLV-I gag protein.

The prevalence of antibodies to HRES-1 peptides pep14–24 and pep117–127 was determined in normal blood donors and patients with immunological disorders. Sera of patients with MS, PSS, SLE, and SJS contained significantly higher HRES-1 peptide binding activity than sera of normal donors. Sera of patients with ECG and AIDS showed no higher binding activity to HRES-1 peptides than sera of normal donors. This suggested that HRES-1 is an autoantigen for patients with the autoimmune diseases MS, PSS, SLE, and SJS. By contrast, patients with AIDS do not have HRES-1 antibodies. Twenty-nine percent of patients with MS, 23% of patients with PSS, 21% of patients with SLE, 10% of patients with SJS, 5% of patients with ECG, and none of the patients with AIDS were considered HRES-1 seropositive. One out of 65 (1.5%) normal blood donors was also HRES-1 seropositive. While HRES-1 seropositivity is most prevalent in patients with MS, it cannot be considered specific for this autoimmune disorder. Autoantibodies to Sm and La antigens are most prevalent in SLE and SJS, respectively (25). La antibodies are not specific for SJS and can also be detected in other autoimmune diseases. Sm antibodies are specific for SLE; however, their prevalence is only 20–30%. This is similar to the prevalence of anti-HRES-1 reactivity in SLE.

There may be two possible mechanisms for generation of HRES-1-specific autoantibodies. First, molecular mimicry—that is, infection by an exogenous retrovirus with cross-reactive epitopes—may trigger HRES-1 antibodies (29, 30). Second, abnormal immunological presentation may lead to autoimmune response against HRES-1. Under normal conditions, intracellular antigens, like HRES-1, are not immunologically presented and may avoid generation of thymic tolerance (25). Autologous proteins may trigger an immune response upon presentation in large quantities, usually accompanying extensive tissue destruction (31, 32). Alternately, overexpression or mutation of HRES-1 may also lead to an aberrant immune response. Involvement of an ERS would explain both the familial aggregation (25) and the presence of antiretroviral protein antibodies in patients with MS and SLE (6–9).

HRES-1 has been mapped to a common fragile site of chromosome 1 at 1q42 (24). This chromosomal localization is interesting in view of a 5-azacytidine-inducible common fragile site at 1q42 (33). This 5-azacytidine inducible fragile site has shown an evolutionary conservation in man, gorilla, and chimpanzee (34), similar to the appearance of HRES-1 in Old World monkeys (23). Furthermore, 5-azacytidine is known to induce endogenous retroviral genes in chicken cells (35). All these data suggest the potential involvement of HRES-1 in the fragility of 1q42. This possibility is particularly interesting in regard to an increased chromosomal fragility in patients with PSS and SLE (36, 37) and the presence of HRES-1 autoantibodies in this subset of autoimmune patients. Chromosomal instability at 1q42 may be associated with aberrant activation and generation of HRES-1 autoantibodies. Availability of recombinant DNA and antibody reagents will help in further elucidating the role of HRES-1 in autoimmune diseases.

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1. Krieg, A. M. & Steinberg, A. D. (1990) *J. Autoimmun.* **3**, 137–166.
2. Abraham, G. N. & Khan, A. S. (1990) *Clin. Immunol. Immunopathol.* **56**, 1–8.
3. Query, C. C. & Keene, J. D. (1987) *Cell* **51**, 211–220.
4. Maul, G. G., Jimenez, S. A., Riggs, E. & Ziemnicka-Kotula, D. (1989) *Proc. Natl. Acad. Sci. USA* **86**, 8492–8496.
5. Kohsaka, H., Yamamoto, K., Fujii, H., Miura, H., Miysasaka, N., Nishioka, K. & Miyamoto, T. (1990) *J. Clin. Invest.* **85**, 1566–1574.
6. Phillips, P. E., Johnston, S. L., Runge, L. A., Moore, J. L. & Poiesz, B. J. (1986) *J. Clin. Immunol.* **6**, 234–241.
7. Koprowski, H., DeFreitas, E. C., Harper, M. E., Sandberg-Wollheim, M., Sheremata, W. A., Robert-Guroff, M., Saxinger, C. W., Feinberg, M. B., Wong-Staal, F. & Gallo, R. (1985) *Nature (London)* **318**, 154–160.
8. Ohta, M. K., Mori, F., Nishitani, H. & Saida, T. (1986) *J. Immunol.* **137**, 3440–3443.
9. Ranki, A., Johansson, E. & Krohn, K. (1988) *N. Engl. J. Med.* **318**, 448–449.
10. Bangham, C. R. M., Nightingale, S., Cruickshank, J. K. & Daenke, S. (1989) *Science* **246**, 821.
11. Richardson, J. H., Wucherpfennig, K. W., Endo, N., Rudge, P., Dalgleish, A. G. & Hafler, D. A. (1989) *Science* **246**, 821–824.
12. Perl, A., Nagy, K., Pazmany, T., Isaacs, C., Baraczka, K., Szabo, T. & Feher, J. (1990) *Arch. Neurol.* **47**, 1061–1063.
13. Chen, I. S. Y., Haislip, A., Myers, L. W., Ellison, G. W. & Merrill, J. E. (1990) *Arch. Neurol.* **47**, 1064–1065.
14. Baltimore, D. (1985) *Cell* **40**, 481–482.
15. Martin, M. A., Bryan, T., Rasheed, S. & Kahn, A. S. (1981) *Proc. Natl. Acad. Sci. USA* **78**, 4982–4986.
16. Bonner, T. I., O'Connell, C. & Cohen, M. (1982) *Proc. Natl. Acad. Sci. USA* **79**, 4709–4713.
17. Callahan, R., Drohan, W., Tronick, S. & Schlom, J. (1982) *Proc. Natl. Acad. Sci. USA* **79**, 5503–5507.
18. Noda, M., Kurihara, M. & Takano, T. (1982) *Nucleic Acids Res.* **10**, 2865–2879.
19. Kroger, B. & Horak, I. (1987) *J. Virol.* **61**, 2071–2075.
20. Harada, F., Tsukada, N. & Kato, N. (1987) *Nucleic Acids Res.* **15**, 9153–9162.
21. Mager, D. L. & Henthorn, P. S. (1984) *Proc. Natl. Acad. Sci. USA* **81**, 7510–7514.
22. Maeda, N. (1985) *J. Biol. Chem.* **260**, 6698–6709.
23. Perl, A., Rosenblatt, J. D., Chen, I. S. Y., DiVincenzo, J. P., Poiesz, B. J., Bever, R. & Abraham, G. N. (1989) *Nucleic Acids Res.* **17**, 6841–6854.
24. Perl, A., Isaacs, C. M., Eddy, R. L., Byers, M. G. & Shows, T. B. (1991) *Genomics* **11**, 1172–1173.
25. Samter, M., Talmage, D. W., Frank, M. M., Austen, K. F. & Claman, H. N. (1988) *Immunological Diseases* (Little, Brown, Boston).
26. Towbin, H. H., Staehelin, T. & Gordon, J. (1979) *Proc. Natl. Acad. Sci. USA* **76**, 4350–4354.
27. Rabson, A. B., Hamagishi, Y., Steele, P. E., Tykocynski, M. & Martin, M. A. (1985) *J. Virol.* **56**, 176–182.
28. Seiki, M., Hattori, S., Hirayama, Y. & Yoshida, M. (1983) *Proc. Natl. Acad. Sci. USA* **80**, 3618–3622.
29. Fujinami, R. S., Oldstone, M. B. A., Wroblewska, Z., Frankel, M. E. & Koprowski, H. (1983) *Proc. Natl. Acad. Sci. USA* **80**, 2346–2350.
30. Fujinami, R. S. & Oldstone, M. B. A. (1985) *Science* **230**, 1043–1045.
31. Griffiths, M. M., Eichwald, E. J., Martin, J. H., Smith, C. B. & DeWitt, C. W. (1981) *Arthr. Rheum.* **24**, 781–789.
32. Reuter, R. & Luhrmann, R. (1986) *Proc. Natl. Acad. Sci. USA* **83**, 8689–8693.
33. Sutherland, G. R., Parslow, M. I. & Baker, E. (1985) *Hum. Genet.* **69**, 233–237.
34. Schmid, M., Ott, G., Haaf, T. & Scheres, J. M. J. C. (1985) *Hum. Genet.* **71**, 342–350.
35. Groudine, M., Eisenman, R. & Weintraub, H. (1981) *Nature (London)* **292**, 311–317.
36. Emerit, J. (1980) *Z. Rheumatol.* **39**, 84–90.
37. Rittner, G., Schwanitz, G., Baur, M. P., Black, C. M., Welsh, K. I., Kuhnl, P. & Rittner, C. (1988) *Hum. Genet.* **81**, 64–70.