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ANTIBODIES TO HUMAN T CELL LEUKAEMIA VIRUS-ASSOCIATED MEMBRANE ANTIGENS IN HAEMOPHILIACS: EVIDENCE FOR INFECTION BEFORE 1980

BRUCE L. EVATT, SIDNEY F. STEIN, DONALD P. FRANCIS, DALE N. LAWRENCE, MARY F. MCLANE, J. STEVEN MCDOUGAL, TUN H. LEE, THOMAS J. SPIRA, CIRILO CABRADILLA, JAMES I. MULLENS, MYRON ESSEX

Centers for Disease Control, Atlanta, Georgia; Emory University, Atlanta, Georgia; and Harvard School of Public Health, Boston, Massachusetts

Summary Human T cell leukaemia virus (HTLV), HTLV proviral DNA, and antibodies to HTLV or a related agent have recently been detected in patients with acquired immunodeficiency syndrome (AIDS). Antibodies against HTLV-related antigens were assayed by means of indirect living cell immunofluorescence of HTLV-infected cells in sera recently collected from Georgia haemophiliacs and in sera collected between 1976 and 1981 from New York haemophiliacs. 5 of 45 Georgia haemophiliacs and 8 of 48 New York haemophiliacs had antibodies to HTLV-associated cell membrane antigen (HTLV-MA). None of the control Georgia patients on haemodialysis or with chronic hepatitis had detectable antibodies. The 5 haemophiliac patients from Georgia with HTLV-MA had significantly fewer T_4 lymphocytes than similar HTLV-MA-negative patients. There were no other significant immunological differences between these groups. These data suggest that transfusions with blood products may expose haemophiliacs to a substantial risk of acquiring HTLV or a related virus.

Introduction

HUMAN T cell leukaemia virus (HTLV), first isolated from a North American in 1980, was subsequently described in Japanese adults with T-cell malignancies and, in symptom-free residents in restricted areas of Southern Japan and the Caribbean basin.[1-7] In these areas, which are apparently endemic for HTLV, 3% to 37% of the healthy adult population may have HTLV antibodies. Less than 1% of healthy adults in selected areas of the USA, Western Europe, and Northern Japan are HTLV seropositive.[3,4,7-9]

More recently, HTLV has been isolated from peripheral-blood T lymphocytes of an American with acquired immunodeficiency syndrome (AIDS),[10] HTLV proviral DNA has been detected in the peripheral-blood lymphocytes of two AIDS patients,[11] and a retrovirus related to HTLV has been isolated from lymph-node cells of a patient with the AIDS-related lymphadenopathy syndrome (LAS).[12] In addition, antibodies to antigens expressed on the cell-surface of HTLV-infected lymphocytes (HTLV-MA) were detected by indirect immunofluorescence in the sera of 25–36% of AIDS patients and 26–30% of patients with LAS. In contrast, only about 1% of matched homosexual controls or blood donors from the mid-Atlantic states had detectable antibody.[13] HTLV apparently establishes a latent-type infection so that individuals who are antibody-positive are likely to remain positive for life.

AIDS has now been seen in four clearly identifiable groups: homosexual and bisexual men, intravenous drug abusers, Haitians, and haemophiliacs.[14] Individuals in this last group are also at risk for infection with viral agents transmitted by blood products (hepatitis B or non-A/non-B hepatitis). Although the mode of transmission of HTLV is unknown, isolation of the virus from peripheral-blood lymphocytes suggests that blood transfusion may lead to its dissemination.[15-17] Because of the risk that patients with LAS or AIDS might transmit HTLV, we decided to examine patients with haemophilia for evidence of infection with HTLV.

Methods

Serum samples were obtained from 45 haemophiliac patients (age 18 to 60), from Georgia enrolled in the Hemophilia of Georgia home care treatment programme and who volunteered to participate. Similarly, samples were obtained from 21 patients on chronic haemodialysis, who received transfusions of blood collected in the metropolitan Atlanta area, and from 29 unselected patients with chronic active hepatitis. All samples were obtained after patient gave informed consent. Serum was separated from blood cells and frozen at −70°C for up to 3 months before assay. Additionally, we obtained frozen serum samples collected between 1976 and 198[illegible] from 47 haemophiliacs living in New York City. This investigation was performed by a protocol approved by the Human Investigation Committee of Emory University.

Antibodies to HTLV membrane antigens (anti-HTLV-MA) were measured by means of indirect living cell immunofluorescence of HTLV-infected cells.[5,8] Two reference HTLV-infected cell cultures, Hut 102[1] and MT 2,[18] were used to detect antibody while 2 HTLV-uninfected human lymphoid lines (8402, a T-cell line,[1] and NC37, an HTLV negative, Epstein-Barr-virus-positive B cell line) served as controls. Samples were judged to be positive (or weakly positive) when they caused fluorescence ⩾50% (or ⩾40%) of the target cells. Each sample was examined at a 1 to 4 dilution by a procedure described elsewhere.[8]

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Mononuclear cells were separated by 'Ficoll-Hypaque' (LSM Solution, Litton Bionetics, Kensington, MD) density gradient centrifugation. T lymphocyte subpopulations were measured by indirect immunofluorescence with commercial monoclonal antibodies (OKT3 for T cells, OKT4 for T helper/inducer cells, and OKT8 for T suppressor/cytotoxic cells, Ortho Diagnostics, Raritan, NJ) and a fluorescein-conjugated anti-mouse immunoglobulin (CDC), on a fluorescence activated cell sorter (FACS IV, Becton-Dickinson, Sunnyvale, CA).[20] B lymphocytes were measured by a peroxidase staining method with a peroxidase-conjugated anti-human immunoglobulin (Cappel Laboratories, West Chester, PA).[21]

Lymphocyte transformation responses were measured by a micromethod with the mitogens phytohaemagglutinin (PHA-P; Difco Laboratories, Detroit, MI), concanavalin A (Con-A; Calbiochem, LaJolla, CA), and pokeweed mitogen (PWM; Gibco, Grand Island, NY).[22] Immunoglobulins G, A, and M were assayed by radial immunodiffusion (Kallestad Laboratories Incorporated, Austin, TX). β_2-microglobulin determinations were performed by a competitive radioimmunoassay (Phadebas, Pharmacia Diagnostics, Uppsala, Sweden). The staphylococcal binding assay (SBA) and the ^{125}I-Clq binding assay (Clq) were performed as described elsewhere.[23,24]

Serum specimens were tested for antigens of, or antibody to, the following microorganisms by methods described elsewhere:[25] cytomegalovirus (CMV) and herpes simplex viruses (HSV) types 1 and 2 (complement fixation [CF] and indirect haemagglutination [IHA]); Epstein-Barr virus (EBV) (antibodies to viral capsid [VCA], nuclear [EBNA], and early antigens [EA]); hepatitis B virus (HBV) (surface antibody [HBsAb] and antigen [HBsAg]), and antibody to the surface and core antigens (anti-HBs and anti-HBc, respectively) (Abbott Laboratories, North Chicago, IL); hepatitis A virus (anti-HAV) (radioimmunoassay, Abbot Laboratories, North Chicago, IL).

Data were analysed by a two-tailed Wilcoxon rank-sum test.

Results

When the criterion for anti-HTLV-MA positivity was fluorescence of ≥50% of the target cells, sera from 2 of 45 Georgia haemophiliacs (4%) and 5 of 48 New York haemophiliacs (10%) were positive; when the criterion for positivity was fluorescence of ≥40% of the cells, another 3 Georgia patients (11%) and 8 New York patients (17%) were positive for anti-HTLV-MA. Seropositivity was evident in the New York samples as early as July, 1978. 39 of the 48 samples and all 8 of the positive samples were collected in 1978 and 1979. No patient in either of the two control groups (chronic haemodilaysis or chronic active hepatitis) had a positive serum.

Abnormalities of the immune system were noted in all 5 Georgia patients who were anti-HTLV-MA positive (table I). All had T-lymphocyte helper-suppressor ratios of 1·0 or less and of these, 3 were markedly depressed. When T-cell subsets of the antibody-positive haemophiliacs were compared with those who were negative, only the T-helper subset was significantly reduced ($p<0\cdot05$) (table II). All 5 antibody-positive patients had an increase in circulating immune complexes; 3 had a rise of either IgG or IgA; and β_2-microglobulin was increased in all 4 patients tested. PWM stimulation was significantly depressed ($p=0\cdot0043$). This depression was characteristic of Georgia haemophiliacs whether or not they were positive for anti-HTLV-MA. All 5 had anti-HBs and anti-HBc, but raised titres to CMV, HSV_1, HSV_2, and EBV were not uniformly present.

TABLE I—IMMUNOLOGICAL EVALUATION OF ANTI-HTLV-MA POSITIVE HAEMOPHILIACS

—	Normal values	Pt A	Pt B	Pt C	Pt D	Pt E
Age (yr)		42	31	32	23	31
Absolute lymph/mm^3	1071–2688	1855	1961	1794	762	1584
T_4/T_8 ratio	1·0–4·0	0·97	0·21	1·0	0·76	0·48
Absolute T_3/mm^3	867–1897	1113	1412	1256	576	1156
Absolute T_4/mm^3	468–1433	668	275	592	253	396
Absolute T_8/mm^3	192–726	686	1255	592	330	824
HBsAg		–	–	–	–	+
Anti-HBs		+	+	+	+	+
Anti-HBc		+	+	+	+	+
SBA* (μg Agg-IgG per ml)	≤34	250	250	48	263	368
Clq (% pre-cipitated)	≤8	17	23	10	22	10
CMV titre		1:16,000	1:256	1:16	<1:8	1:16,000
HSV1 titre		1:32	<1:8	1:16	<1:8	1:1024
HSV2 titre		1:16	<1:8	1:8	<1:8	1:64
EBV titre		1:200	1:50	1:100	1:100	1:100
β_2-microglo-bulin (μg/dl)	<2200 μg/dl (<40 yr) <3000 μg/dl (≥40 yr)	3234	Not done	2415	4536	5292
IgG (mg/dl)	617–1325	1880	1300	1060	2280	2010
IgA (mg/dl)	65–301	232	57	195	395	544
IgM (mg/dl)	26–223	153	140	90	142	41
PHA (% of reference)	75–155	92	96	108	150	79
Con A (% of reference)	43–218	255	123	133	229	137
PWM (% of reference)	37–187	51	0	18	42	26

*Staphylococcal binding assay.

TABLE II—COMPARISON OF T CELL POPULATION IN ANTI-HTLV-MA POSITIVE AND NEGATIVE GEORGIA HEMOPHILIACS

	HTLV-MA negative (N=42)		HTLV-MA positive (N=5)		
	Median	Range	Median	Range	p
T_4/T_4 ratio	0·935	0·3–1·87	0·76	0·21–1·0	0·20
Absolute T/mm^3	1380	625–4555	1156	576–1412	0·28
Absolute T_4/mm^3	652	252–1891	396	253–668	<0·05
Absolute T_8/mm^3	739	323–3089	686	330–1255	0·79

Discussion

The prevalence of a marker for HTLV infection in our sample of patients with haemophilia is greater than that expected in the normal US population[13] or that found in a group of haemodialysis patients or in a group of chronic active hepatitis patients. Haemophiliacs seem to have a substantial risk of exposure to HTLV or a related virus. Chronic renal dialysis patients in Georgia receive transfusions from single local donors. Transfusion with factor VIII concentrate, prepared from pools of plasma obtained from 2000 to 20 000 donors may be the factor responsible for exposure of haemophiliacs to an infectious agent.

The transfusion history of Georgia haemophiliacs from 1980 through 1982 shows no difference in the total number of factor VIII units transfused into HTLV-positive patients and into HTLV-negative patients. However, the number of factor VIII units received by 4 of the 5 HTLV-positive patients was at or above the median amount received by the HTLV-negative patients during those 3 years. The number of patients studied was small. In addition the observation period

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chosen for factor VIII administration was arbitrary and limited to three years and thus may not reflect the true risk of infection by an HTLV-related agent. Either of these factors may be responsible for the inability to demonstrate a statistical association between factor VIII usage and the presence of antibodies to an HTLV-related agent.

Comparison of the current prevalence of HTLV seropositivity in Georgia haemophiliacs with that found 4 years earlier in New York haemophiliacs shows no upward trend. The risk for haemophiliacs acquiring an HTLV-related infection may not have increased significantly during the past 4 years. However, comparisons between these geographically and temporally separated groups of haemophilia patients may not be valid. It is also possible that, if factor VIII is a risk factor for infection with HTLV-related virus, the risk of HTLV in sources of plasma used to prepare factor VIII concentrate may not have changed much during recent years.

The Public Health Service recommends that individuals at high risk for developing AIDS should not donate blood.[26] Our study suggests that a virus similar to or identical with one associated with the development of a lymphoproliferative disorder or an immunosuppressed state may be transmitted in a manner similar to AIDS, and thus the likelihood of the plasma pool from donors contributing to factor VIII transmitting both AIDS and an HTLV-like virus seems to be quite high. HTLV may spread from person to person by very close contact;[27] the implications of this kind of dissemination for haemophiliacs and their families remain to be determined.

Antibodies to HTLV-MA have recently been detected in up to 60% of the patients with AIDS. These antibodies appear to be specific for HTLV or a related agent. Sera which are strongly positive precipitate the core HTLV protein, p24[13] (Essex M, et al, unpublished). However, the true prevalence of detectable antibodies in patients with AIDS, LAS, and haemophilia may be underreported. The test may lack sensitivity, the criterion for positivity may be too stringent, or the test may be detecting a cross-reacting antigen or an agent which is similar to but not identical with HTLV. Were this last possibility true, our test would not be able to discriminate between a true HTLV infection and any cross-reacting agent that may cause AIDS.

An aetiological association between HTLV-related agents and AIDS has been suggested. The presence of HTLV antibodies in a significant proportion of haemophiliacs since 1978 raises the question of why AIDS has not yet been detected in these patients. Several explanations are possible: (1) HTLV-related agents do not cause AIDS. (2) The HTLV antibodies measured by this test detect a specificity present on more than one agent, including a variant strain of HTLV[12,28] that might differ from those previously found in Southern Japan and the Caribbean. These populations have almost certainly been infected with an HTLV-related virus for at least several years before 1980. (3) An HTLV-related virus could be involved but by itself may be insufficient to produce the AIDS clinical syndrome. (4) The latent period for AIDS in haemophiliacs may be longer than previously suspected. (5) Some haemophiliacs may already have, or have had, subclinical AIDS.

While there are only 5 anti-HTLV-MA positive Georgia patients, a significant difference in absolute T_4 numbers was observed when they were compared with anti-HTLV-MA-negative patients. No other immunological or serological differences were observed between the 2 groups. The difference in absolute T_4 numbers was significant only at the 0·05 level, and further study will be required for corroboration. This observation may be important because HTLV preferentially infects T_4 lymphocytes and reduction in T_4 lymphocytes is almost universal in AIDS cases.[15]

The importance of finding HTLV-like viral infections in haemophilia patients and patients with and without AIDS or LAS[10-13] remains uncertain. An intensive longitudinal study of individuals at high risk for developing AIDS will be required to determine the possible aetiological relation between an HTLV-like virus and AIDS.

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Correspondence should be addressed to B. L. E., Division of Host Factors, Centers for Disease Control, 1600 Clifton Road, 1-1407, Atlanta, GA 30333.

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THE FETUS AS AN ALLOGRAFT: EVIDENCE FOR PROTECTIVE ANTIBODIES TO HLA-LINKED PATERNAL ANTIGENS

D. A. POWER G. R. D. CATTO
R. J. MASON A. M. MACLEOD
G. M. STEWART K. N. STEWART
W. G. SHEWAN

Department of Medicine, University of Aberdeen, and Blood Transfusion Service, Aberdeen Royal Infirmary

Summary Non-cytotoxic antibodies to paternal B lymphocytes were detected in sera from 11 of 11 multiparous women and from 11 of 16 normal primigravidae during the first trimester of pregnancy. These antibodies were not, however, detected in sera from 9 of 10 women of comparable gestation at the time of spontaneous abortion. By means of a rosette inhibition assay, the difference in antibody activity between the primigravidae (mean 37·9±19%, median 36·5%) and the women subject to spontaneous abortion (mean 7·3±11·6%, median 0%) was statistically significant. This antibody activity was not directed to the known HLA specificities (HLA—A, B, C, or DR), but linkage to the HLA gene complex was suggested by family studies. These results provide evidence for an HLA-linked antigen system not defined by conventional tissue-typing techniques. Fetomaternal disparity at this antigenic site may be important for successful pregnancy.

Introduction

THE survival of the fetal allograft remains a central immunological puzzle. There is considerable evidence of maternal cellular[1] and humoral[2] immune responses to the fetus but, paradoxically, this may be essential for a successful outcome.[3,4] Indeed, parental sharing of HLA antigens may be associated with recurrent spontaneous abortion.[5] Although antibody blocking factors have been described in various in-vitro systems,[4,6] these have not been detected in early pregnancy, when their presence is thought necessary to prevent abortion.[7] Non-cytotoxic Fc-receptor-blocking antibodies have been detected in pre-transplant sera from some renal-allograft recipients, and their presence correlated with improved graft survival.[8,9] Our aims in the present study were (a) to detect non-cytotoxic Fc-receptor-blocking antibodies directed against paternal B lymphocytes in sera from normal first-trimester primigravidae and (b) to determine whether these antibodies were directed against an HLA-coded antigen.

Patients and Methods

Maternal sera were obtained from 10 patients undergoing a first-trimester spontaneous abortion, 16 normal primigravidae, and 11 multiparae at varying times of gestation and up to 15 years after pregnancy. Serum samples were obtained from the first group at the time of abortion (mean 10·9 weeks' gestation), and all but 1 had experienced multiple abortions (median 2, range 1–4). None of this group had ever had a normal pregnancy. Blood was obtained from normal primigravidae during the first trimester (mean 10·6 weeks' gestation). Additional serum samples were available from 7 primigravidae during early pregnancy and from 4 of these before conception.

All sera were ultracentrifuged (100 000 *g* for 90 min) to remove immune complexes.

Preparation of IgG and F(ab¹)2 Fragments from Maternal Serum

Serum was dialysed against 0·015 *M* phosphate buffer (pH 7·0) and fractionated on a diethylaminoethyl-cellulose (Whatman DE-52) column equilibrated with the same buffer. The break-through peak was collected and concentrated back to the starting volume. This material was dialysed against phosphate-buffered saline and tested for purity by immunoelectrophoresis.

F(ab¹)2 fragments of a second IgG preparation were prepared by pepsin digest (2 h at 37°C) and isolated after fractionation on 'Sephadex G150'. Some remaining whole IgG, detected by sodium-dodecyl-sulphate/polyacrylamide-gel electrophoresis, was absorbed with formaldehyde-treated *Staphylococcus* A (Cowan strain).

Platelet Absorption of Maternal Sera

A sample of serum was absorbed with platelets pooled from more than 100 donors. The serum was incubated for 1 h at 37°C and for 10 h at 4°C with an equal volume of platelets.

Target Lymphocytes

Target lymphocytes used in this study were (a) paternal T and B lymphocytes, (b) B lymphocytes from paternal families, and (c) B lymphocytes from a panel of normal unrelated subjects. T and B lymphocytes, prepared by means of 'Ficoll'/sodium-diatrizoate density-gradient centrifugation of heparinised peripheral blood (30 ml), were separated on nylon-wool columns.[10] Target-cell populations usually showed more than 85% viability if cryopreserved and more than 95% if freshly prepared (ethidium-bromide/acridine-orange dye exclusion). Over 75% of B lymphocytes were positive for surface-membrane immunoglobulin; more than 90% of T lymphocytes were E-rosette positive.

Alloantibody Assays

1. EA rosette inhibition (EAI) was carried out with a modification of a microrosette technique.[11] Erythrocyte-antibody complexes (EAs) were made by incubating, without complement, washed ox erythrocytes with rabbit anti-ox-erythrocyte antibodies. B lymphocytes were incubated with test serum, or with ultracentrifuged fetal-calf serum (FCS) or normal human serum as control, in the wells of a microtitre plate for 45 min at 4°C.

The cells were then washed and incubated overnight at 4°C with 2% ox EAs. The cells were resuspended, and between 200 and 400 lymphocytes were counted, under simultaneous ultraviolet light and phase-contrast illumination, in a Neubaur counting-chamber with an acridine-orange-coated coverslip. The percentage of rosette-forming cells was determined. A rosette was defined as three or more erythrocytes/lymphocyte. Control cell suspensions contained 30–60% rosetting cells. The percentage EAI was calculated from the formula:[8]

$$\%\ \mathrm{EAI} = 100\left(1 - \frac{\%\ \text{EA rosettes in test serum}}{\%\ \text{rosettes in control FCS}}\right)$$

2. Complement-dependent lymphocytotoxicity was determined with standard microlymphocytotoxicity assays against T and B cells.[12] B-lymphocyte assays were done at 5°C, 23°C, and 37°C.

Statistical Methods

The difference in median EA inhibiting activity between primigravidae and women subject to spontaneous abortions was assessed with the Wilcoxon rank sum test; the level of significant inhibition in a particular test serum was accepted as a difference between control and test rosetting values at the $p<0·05$ level (chi-squared test with Yates' continuity correction). Family studies were analysed with the lod score method.

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