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HTLV-I Antibody Status in Hemophilia Patients Treated with Factor Concentrates Prepared from U.S. Plasma Sources and in Hemophilia Patients with AIDS

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Key words

HTLV – Hemophilia – Factor concentrates

Summary

Serum samples from 85 Austrian hemophilia patients treated with lyophilized factor concentrates prepared from U.S. plasma sources, 24 hemophilia patients from Georgia on a home therapy program with factor concentrates, and 10 U.S. hemophilia patients with acquired immunodeficiency syndrome (AIDS) were analyzed by two different methods for the presence of antibodies to the major internal antigen of human T-cell leukemia virus I (HTLV-I) p24. All but one, a Georgia sample, were negative. The absence of antibody to HTLV-I p24 in the serum of European hemophilia patients, of U.S. hemophilia patients with no symptoms of AIDS, and of U.S. hemophilia patients with AIDS is interpreted as an indication of the lack of ready transmissibility of HTLV-I in lyophilized factor concentrates.

Introduction

Concern has been expressed that the first isolated subtype of human T-lymphotropic retrovirus (HTLV-I) may be transmissible by blood or blood derivatives (1–3). Evidence to support the hypothesis of transmission of HTLV-I by blood transfusion with whole blood or cellular blood components has been reported (4). Because of the endemicity of HTLV-I in parts of the Western hemisphere (5, 6) and because of concern that HTLV-like viruses may be transmitted in American or other blood products (4, 7, 8), we evaluated serum of hemophilia patients for evidence of antibodies to HTLV-I. The patient groups studied included U.S. hemophilia patients with the acquired immunodeficiency syndrome (AIDS), European hemophilia patients treated with lyophilized factor concentrates manufactured from U.S. plasma sources, and U.S. hemophilia patients from the Southeastern United States who have no symptoms of AIDS. This is an area of known HTLV-I endemicity (9).

Materials and Methods

Patients

Serum samples were collected from hemophilia patients in Austria, asymptomatic U.S. hemophilia patients, and hemophilia patients with AIDS. The Austrian specimens were collected between August 1982 and June 1983 in Vienna from 81 patients with hemophilia A (4 mild, 4 moderate, 73 severe) whose ages ranged from 6 to 72 years, median 24, and 4 patients with hemophilia B (2 moderate, 1 severe, 1 undetermined severity) whose ages ranged from 8 to 56 years. All 81 patients had been treated with factor VIII or IX concentrates in the hemophilia treatment center at the University of Vienna for several years. All patients with hemophilia A had been treated with intermediate-purity factor VIII concentrate produced in Vienna (Kryobulin, Immuno AG). Kryobulin is a lyophilized preparation, manufactured from a large donor pool containing a variable (10%–90%) proportion of plasma from U.S. donors. From August 1982 to April 1983, 38 of the patients were treated with this same preparation, while 30 received another intermediate-purity factor VIII concentrate preparation produced by Schwab Co., Vienna. Starting in April 1983, some of the patients who had developed immunological abnormalities were treated with a highly purified heat-inactivated preparation produced by Behring Co., West Germany. The mean consumption of factor VIII concentrate of all patients in the year prior to April 1983 was 55,000 U/year (range 0 to 456,000, median 45,000), seven patients with inhibitors having been treated only with activated prothrombin complex preparations in the year. Patients with hemophilia B received Bebulin (Immuno AG, Vienna), a factor IX preparation manufactured from the same sources as Kryobulin, and required an average of 43,000 U/year (range 30,000–56,000). Immunologic evaluation of the majority of the Austrian participants has been presented in detail elsewhere (10).

The U.S. serum samples were collected in November/December 1982 and again in August 1983/January 1984 from 24 hemophilia A patients (ages 16 to 59 years, median 31) enrolled in the Hemophilia of Georgia home care treatment program. These patients had been treated with lyophilized factor concentrates manufactured from U.S. sources only. Serum was separated from blood cells and frozen at −70° C for up to 6 months before assay.

Serum samples were also collected from October 1983 to January 1984 from 10 U.S. patients with hemophilia A and AIDS (ages 10 to 53 years, median 27.5). None of these patients had a known risk factor for developing AIDS other than hemophilia. Three of these patients received no blood product other than factor concentrates in the 5 years prior to their diagnosis.

For control purposes serum samples were assayed by both methods described below for HTLV-I p24 from 29 Georgia patients with chronic active hepatitis (age range 21 to 73 years, median 39.5) and 21 Georgia patients receiving chronic hemodialysis (age range 24 to 76 years, median 59), a population repeatedly given transfusions of blood collected in the Atlanta metropolitan area. All of these 50 serum samples had been found to be negative for antibody to membrane antigens on cells infected with HTLV-I (HTLV-MA) (11).

Immunologic Methods

Serum was examined for antibodies to the major core protein of HTLV-I (p24) by two methods. The first, described elsewhere (12), was a radioimmune precipitation of ^{125}I-labeled HTLV-I p24 by human serum. Briefly, ^{125}I-labeled HTLV-I p24 (8000–10000 cpm) was incubated with serial 1:1 dilutions of the human serum in buffer. After 2 hr at 37° C, the incubation was continued overnight at 4° C. A predetermined amount of inactivated *Staphylococcus aureus* cells (Pansorbin, Calbiochem) was then added and the reaction mixture was diluted with the buffer. After further

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Table 1 Presence of antibody to HTLV-p24 by immune precipitation and by Western blot technique, by patient category

Category	HTLV p24	
	radioimmune precipitation	Western blot technique
Austrian hemophilia patients	0/85[1])	0/85
U.S. hemophilia patients without AIDS	1/24	1/24
U.S. hemophilia patients with AIDS	0/10	0/10
Chronic active hepatitis patients	0/29	0/29
Hemodialysis patients	0/21	0/21

[1]) Ratio indicates number positive of total number tested.

incubation for 1 hr at 37° C, followed by 2 hr at 4° C, the precipitates were collected by centrifugation, and the radioactivity in the precipitates was determined. The serum from a known positive control patient precipitated >90% of the labeled p24.

The second assay for antibody to HTLV-I p24 was a sodium dodecyl sulfate/polyacrylamide gel electrophoresis (SDS-PAGE) and enzyme-linked immunoelectrotransfer blot (Western blot) technique described elsewhere (13) using HTLV-I purified from a T-cell line infected with HTLV-I (MT-2) (14). Details relevant to the present application are the following: SDS (final concentration 1%) and 2-mercaptoethanol (final concentration 5.0%) were added to purified MT-2 virus suspension. The preparation was heated at 65° C for 30 min and diluted 1:100 in sample buffer (1% SDS, 10% glycerol, 0.25 µg/ml bromphenol blue, 0.01 M Tris buffer [pH 8.0]). A 150 µl sample volume was electrophoresed on 3.3%–20% gradient acrylamide gels. PAGE-resolved proteins were electrophoretically transferred to nitrocellulose sheets (60 V × 3 hr, 4° C). The washed sheets were cut into strips and incubated overnight at 4° C with 1:100 dilutions of test serum. The strips were washed, incubated for 2 hr at room temperature with a peroxidase-conjugated anti-human Ig reagent, and washed again. Color reactions were developed with 3,3'diaminobenzidine and H_2O_2 (13). Positive and negative sera as well as a buffer control were included in all runs.

Results

All serum samples from Austrian hemophilia patients and from five random normal donors were non-reactive by both the Western blot and radioimmune precipitation methods. Significant antibody reactivity to HTLV-I p24 was found in only one Georgia hemophilia patient who had no significant travel history and had received no blood products other than factor concentrates in 10 years. All other Georgia hemophilia serum samples were non-reactive (Table 1). All 10 of the U.S. hemophilia-associated AIDS serum samples were negative for antibody to HTLV-I p24. All the samples from the anti-HTLV-MA-negative hemodialysis and chronic active hepatitis patients were negative for antibody reactivity to HTLV-I p24. Strong antibody reactivities were obtained with previously screened sera with known reactivity to HTLV-I p24. Results obtained by radioimmune precipitation were corroborated by the Western blot technique.

Discussion

The scarcity of antibody positivity to HTLV-I p24 core antigen in U.S. hemophilia patients and the absence of HTLV-I p24 antibody in European hemophilia patients treated with lyophilized factor concentrates prepared in part from U.S. plasma pools indicate a lack of ready transmissibility of HTLV-I in lyophilized factor concentrates. HTLV-I might not be present in the cell-free factor concentrates used as therapy for hemophilia, although isolation of HTLV-I from peripheral blood lymphocytes suggests that cellular blood transfusion may result in retroviral transmission (1, 3, 15). Although there is in vitro evidence that cell-free transmission of HTLV-I to non-lymphoid cell lines may be achievable under certain conditions (16), most attempts to use cell-free HTLV-containing supernatants to infect normal human lymphoid cells have failed (17), suggesting that cell-to-cell contact is important for HTLV-I transmission. This hypothesis is supported by the finding of seroconversion to anti-HTLV-positivity after transfusion in Japan only in those recipients given whole blood or cell components but not with fresh frozen plasma from donors having antibody to HTLV (4). The same may pertain with respect to the European hemophilia patients presented here, whose only known exposures to whole cell transfusions would be to blood from donors in an area non-endemic for HTLV-I.

The absence of evidence of antibody to HTLV-I p24 in the 10 hemophilia patients with AIDS is not surprising. A newly discovered subgroup of retrovirus collectively designated as the lymphadenopathy virus (LAV/HTLV-III) rather than HTLV-I is now believed to be the causative agent of AIDS (18–21). In addition, cell-to-cell contact appears to be important for HTLV-I transmission as noted above, and three of the 10 hemophilia patients with AIDS had no known exposure to blood products other than cell-free factor concentrates in the five years prior to diagnosis.

A comparison of the prevalence of seropositivity of HTLV-I and LAV/HTLV-III in hemophilia patients treated with factor concentrates suggests differing modes of transmission. A recent serosurvey of 25 U.S. hemophilia A patients for evidence of antibody to LAV core protein, p25, and LAV envelope protein, p41, has demonstrated a significant association between seropositivity and the amount of factor VIII concentrate used over a 3-year period (22). These studies indicate that LAV may be transmitted by some blood products and suggest that factor VIII concentrate is implicated as a medium of LAV/HTLV-III transmission. Likewise, epidemiologic data indicate that cell-free factor concentrate can be a vehicle of transmission of the AIDS agent, since several cases of hemophilia-associated AIDS with no other known risk factors have been reported to CDC in which no blood products other than factor concentrates had been taken in the 5 years prior to diagnosis. However, in the data presented here, in a large group of European hemophilia patients treated to a great extent with lyophilized factor concentrates prepared from large donor pools containing a considerable proportion of imported plasma from U.S. donors, the absence of antibody to HTLV-I p24 in serum is an indication of a lack of ready transmissibility of HTLV-I in such cell-free factor concentrates. Several considerations are important in interpreting these findings:

First, it is possible that the U.S. and Austrian sera assayed here for p24 did contain other anti-HTLV-I antibody that was missed because the assays used might detect only anti-p24 antibody, and patients with AIDS or AIDS symptoms may react differently to various HTLV-I antigens. It has been proposed that the absence of HTLV-p24 in some patients having antibody to HTLV-MA is likely to be a consequence of low immunogenicity of the p24 core protein rather than a lack of exposure to HTLV-I (8). The isolation of virus with antigens in common with HTLV-I from two patients with AIDS and negative p24 serology (23) is compatible with this possibility.

Second, our sample sizes of 85 European and 35 U.S. hemophilia AIDS and non-AIDS patients may have been too small to assess the true prevalence of antibody to p24 antigen in these populations. To date, the only other study reporting antibody to HTLV-I p24 in European hemophilia patients (24) has included much smaller numbers. That study, conducted in France, found no evidence of antibodies to HTLV-I p24 by radioimmunoassay in the sera of four patients receiving French cryoprecipitate, two patients receiving American factor concentrates, and 17 patients receiving factor VIII concentrates from French and non-French sources.

Third, the presence of antibody to HTLV-I p24 found in the one Georgia hemophilia patient may simply reflect the low level of endemicity of HTLV-I in the United States (25–27) and manifested perhaps to a greater extent in a population that has undergone occasional transfusions within the United States.

Fourth, it is possible that the absence of antibody to HTLV-I in European hemophilia patients treated with lyophilized factor concentrates manufactured from large U.S. donor pools may reflect a very low prevalence of HTLV-I infection among U.S. plasma donors.

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Comparison of the Interactions of Fibrinogen and Soluble Fibrin with Washed Rabbit Platelets Stimulated with ADP

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Key words

Platelets – Fibrinogen – Fibrin – ADP

Summary

Because fibrin, formed at a site of vessel wall injury, is involved in the formation and stabilization of a platelet aggregate or thrombus, we have studied reactions of fibrin with rabbit platelets. Gly-Pro-Arg-Pro, an inhibitor of fibrin polymerization, was used to prepare soluble fibrin. Fibrin alone did not cause aggregation of washed platelets, but addition of ADP caused aggregation and deaggregation identical to those observed in the presence of fibrinogen. Specific binding of ^{125}I-fibrin to ADP-stimulated platelets was similar to that of ^{125}I-fibrinogen, but ^{125}I-fibrin did not dissociate, even in the presence of high concentrations of apyrase. High non-specific binding of ^{125}I-fibrin was observed that was not associated with aggregation. EDTA, prostaglandin E_1 (PGE_1) and creatine phosphate/creatine phosphokinase prevented ADP-induced aggregation in the presence of fibrin and caused rapid deaggregation when added after ADP. They also inhibited ^{125}I-fibrin binding when added before ADP, and EDTA or PGE_1 caused partial dissociation of bound ^{125}I-fibrin. In vivo, fibrin may bind to stimulated platelets, polymerize, form a gel, and interact with components of the plasma, the platelet aggregate, and the exposed subendothelium.

Introduction

Platelets and fibrinogen both take part in the formation of hemostatic plugs and thrombi at sites of vessel injury and thus play an important role in hemostasis and thrombosis. The endothelium of blood vessels is nonthrombogenic, but upon vessel injury platelets adhere to the exposed subendothelium and release some of their granule contents, and the coagulation pathway is activated, leading to the formation of thrombin. Thrombin plays at least two roles in the formation of thrombi; it activates platelets, causing them to aggregate, and it converts fibrinogen to fibrin which clots and forms an insoluble mesh around the platelets, thus stabilizing the platelet aggregate. Subsequently the platelets are involved in the retraction of the fibrin clot.

Interactions between platelets and fibrinogen have been extensively studied. It has been shown by several groups of investigators that when platelets are activated fibrinogen receptors are expressed on the platelet membrane, fibrinogen binds to the platelets and they aggregate (1–6). Fibrinogen binding appears to be required for platelet aggregation to occur. Platelet-fibrin interactions, which are important in vivo because of the simultaneous formation of fibrin and platelet aggregates at a site of vessel wall injury, have been difficult to study; the insolubility of fibrin causes complications in distinguishing between the primary reaction of soluble fibrin with platelets and the subsequent entrapment of platelets within the insoluble fibrin mesh. To maintain fibrin in a soluble state solvents such as 3 M urea, 1 M NaBr or 1% monochloroacetic acid have generally been used, but none of these can be used in platelet studies. Fibrin also forms soluble complexes with fibrinogen, but the presence of fibrinogen would create difficulties in interpretation in studies of platelet-fibrin interactions. Recently, small peptides have been synthesized that bind to fibrin and prevent it from aggregating to form an insoluble clot (7, 8). We have used one of these peptides, glycylprolylarginylproline (Gly-Pro-Arg-Pro), to prepare soluble fibrin, and have investigated some of its reactions with ADP-stimulated rabbit platelets. The results of our studies are reported in this paper.

Materials and Methods

Materials

Human fibrinogen (grade L) was from AB Kabi, Stockholm, Sweden; fibrinogen that was free of factor XIII was prepared as previously described by a slight modification (9) of the method of Lawrie et al. (10). Human α-thrombin (lot H-1) was kindly supplied by Dr. D. L. Aronson, Bureau of Drugs and Biologics, FDA, Bethesda, MD; it was dissolved at 100 U/mL in 50% glycerol and stored at −20°. Prostaglandin E_1 (PGE_1) was from the Upjohn Co., Kalamazoo, MI; Creatine phosphate (CP), creatine phosphokinase (CPK), ADP, hirudin and heparin (porcine intestinal mucosa, Grade II) were from Sigma Chemical Co., St. Louis, MO. Bovine albumin (Pentex, fraction V) was from Miles Laboratories, Elkhart, IN. Gly-Pro-Arg-Pro was supplied by Vega Biochemicals, Tucson, AZ, and by Peninsula Laboratories, Inc., Belmont, CA. For early experiments, D-phenylalanylprolylarginine-chloromethyl ketone (D-Phe-Pro-Arg-CH_2Cl) was kindly supplied by Dr. E. N. Shaw of Brookhaven National Laboratory, Upton, NY; for later experiments it was obtained from Calbiochem-Behring, San Diego, CA. Antithrombin III (ATIII) was prepared by the method described by Miller-Andersson et al. (11). Apyrase, prepared by the method of Molnar and Lorand (12), was dissolved in 0.15 M NaCl and stored at −20°; at a concentration of 3 μL/mL it caused 35% conversion of 9 μM ADP to AMP in 2 min at 37°; at 0.125 μL/mL we observed 10% degradation of 9 μM ADP in 10 min at 37°. Na^{125}I (carrier free, NEZ 033L) was from New England Nuclear, Boston, MA. Fibrinogen was iodinated with ^{125}I by the method described by McFarlane (13). ^{131}I-albumin was from Charles E. Frosst and Co., Montreal, Canada. ^{14}C-Serotonin was obtained as 5-hydroxytryptamine-3'-^{14}C-creatinine sulfate (54 mCi/mmol) from Amersham Corp., Arlington Heights, IL. An RIA-Quant FPA Test Kit for the determination of human fibrinopeptide A was from Mallinckrodt, Inc., St. Louis, MO. All other chemicals were reagent grade. Reagents were dissolved in 0.15 M NaCl before use, and all concentrations are expressed as final values after all additions.

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