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# Human T-Cell Leukemia Virus (HTLV-I) p24 Antibody in New York City Blood Product Recipients

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Human T-cell leukemia virus (HTLV-I) is known to be associated with certain hematologic malignancies, and a related virus, HTLV-III/LAV, might be the cause of AIDS. Some persons with AIDS have had evidence of HTLV-I infection. Unrelated to these findings, it has been suggested that HTLV-I is transmitted via blood products. We therefore evaluated the serologic status to the HTLV-I core antigen p24 of 48 persons with hemophilia (Hem A) receiving factor concentrate therapy (a group at risk for AIDS), 49 persons with β-thalassemia major (Thal) receiving frozen packed red blood cells therapy (FPRC), 26 patients with sickle cell anemia (SCA) receiving FPRC, and 18 persons not receiving any blood products. All participants were clinically well; only one had a risk factor other than hemophilia for AIDS, and all were from New York City, an area with a high incidence of AIDS. No Hem A or nontransfused persons had serum antibody to HTLV core p24 antigen; three with Thal and one with SCA were antibody-positive. These results were confirmed by both radioimmunoprecipitation and Western blot techniques. Positive serology did not correlate with any immune findings or quantity of blood products used. These data support that HTLV-I is preferentially transmitted through cellular blood products and that it is an infection for which cellular blood product recipients in at least some areas of the United States are at risk. Concentrate products appear free of transmission risk relative to cellular blood products, but we cannot be certain that this safety is absolute. The public health implications of blood product transmission of HTLV-I merit active, long-term investigation.

**Key words: HTLV, AIDS, transfusion, hemophilia, sickle cell anemia, thalassemia**

## INTRODUCTION

Human T-cell leukemia virus (HTLV-I) has been cultured from, and related DNA sequences observed by hybridization in, cells of patients with cutaneous T-cell lymphoma (CTCL) [1,2] or with adult T-cell leukemia (ATL) [1,3–7]. It has only rarely been found in clinically normal individuals who were [7] or were not [8,9] relatives of patients with ATL or CTCL. Similarly, the prevalence of serum antibody specific for a core antigen of HTLV, p24, has been found to be extremely high in patients with ATL or CTCL [10–12] and higher in relatives of these patients than in

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general population controls [10–12]. Reported population prevalences of p24 antibody have varied from less than 1% for U.S. and Japanese populations [10,11] to 3% for a Caribbean population [13]. When antibody to a second core antigen, p19, was also evaluated, researchers found that 0.5% of Washington, DC, participants, 1.9% of Georgia participants, 1.5% of Japanese participants from ATL-nonendemic areas, and 11.9% of Japanese participants from ATL-endemic areas were antibody-positive to at least one core antigen [12]. In that study, 90% of patients with ATL and 39% of these patients' relatives were antibody-positive [12]. These and other data have lead to the hypothesis that HTLV-I in fact has a causal association with certain T-cell neoplasms [13,14].

Researchers have reported finding HTLV-I in cells of persons with the newly recognized acquired immunodeficiency syndrome (AIDS) [15,16], HTLV sequences in the genome of an AIDS patient [17], and HTLV-related membrane-reactive antibodies in a number of AIDS patients [18,19]. All these findings have led to speculation about whether or not HTLV-I represents an infection for which persons at risk of AIDS are also at risk and about whether or not infection with this organism is of importance in AIDS patients [15–20].

Persons with hemophilia are known to be at heightened risk for AIDS [21], with 56 U.S. cases of AIDS in hemophiliacs having been reported to the Centers for Disease Control (CDC) as of November 15, 1984, from an estimated U.S. hemophiliac population of 14,500 [22]. AIDS transmission in these cases is thought to have occurred via the intravenous route, through receipt of contaminated blood products. Further, a heightened prevalence of immune abnormalities similar to those found in AIDS has been seen in clinically asymptomatic hemophiliacs [23–30]. Consistent with the possibility that HTLV-I infection can occur, also via an intravenous route, in this population at high risk for AIDS are the following data. First, serologic evidence of HTLV-related infection has been found in AIDS patients with hemophilia [19]. Second, researches have found a high prevalence of antibody to HTLV-associated antigens in hemophiliacs asymptomatic for AIDS [19,31]. Third, HTLV-I appears to be transmissible through blood products [32] and, in fact, researchers have expressed concern that blood donors in endemic countries should be screened for the presence of antibody to HTLV-I [8,9,32–35]. However, more recently, Japanese researchers have presented laboratory or serologic evidence that HTLV-I is transmissible through fresh cellular blood products but not plasma and presumably, therefore, not through plasma products [36,37].

To help clarify the relationships between HTLV-I, AIDS, and transfusion therapy in the United States, we evaluated the p24 antibody status of cellular and noncellular blood product recipients without AIDS and residing in New York City, a high-risk area for AIDS, and of 18 nontransfused persons from the same area. These results are compared with those published or in press concerning other populations, including hemophiliac AIDS patients.

## PARTICIPANTS

Participants were enrolled in 1983; details of patient selection and evaluation are given in detail elsewhere [38]. Briefly, serum was obtained from participants with hemophilia A (Hem A) who had received $\geqslant$100,000 units of factor VIII concentrate in the 12 years preceding their enrollment from two treatment centers in New York

City. Only seven had received other blood products in the previous 5 years for isolated bleeding incidents. These two centers treat approximately 40% of the hemophiliac patients in that city. We also evaluated 49 persons with β-thalassemia (Thal) from a center that treats 67% of the transfusion-dependent thalassemic patients in New York City. These persons had each received 12–24 units of frozen packed red blood cells (FPRC) per year for the previous 3 years, to maintain a hemoglobin level of $>11$ mg/dl. Three had also received platelets, and one had received plasma at isolated points in time. Twenty-six patients with sickle cell anemia (SCA) were enrolled from three centers in New York City that use routine transfusion therapy. These persons had each received $>6$ units of FPRC per year for the previous 3 years. One had also received plasma at an isolated point in time. Blood specimens were also obtained from 18 healthy New York City health-care personnel who volunteered to be controls for laboratory testing.

## METHODS

The presence or absence of antibody to HTLV-I p24 core antigen was assessed using two techniques. The first was a radioimmune precipitation (RIP) of $^{125}$I-labeled purified HTLV-I p24 by human sera, using a double antibody system. Purification, radiolabeling, and immunoprecipitation techniques are described in detail elsewhere [10,39]. Briefly, p24 antigen was purified from a MT-2 HTLV-I–infected T-cell line and $^{125}$I-labeled HTLV-I p24 (8,000–10,000 cpm) was incubated with human sera in a final volume of 200 μl of 20 mM Tris-HCl, pH 7.5, 200 mM NaCl/liter mM EDTA, 0.3% Triton X-100, 0.1 mM phenylmethylsulfonyl fluoride containing bovine serum albumin 2 mg per ml. After 2 hr at 37°C, the incubation was continued over night at 4°C. A predetermined amount of Staphylococcus aureus cells (Pansorbin, Calbiochem, La Jolla, CA) was then added, and the reaction mixture was diluted to 1 ml with the above buffer. After further incubation for 1 hr at 37°C, followed by 2 hr at 4°C, the precipitates were collected by centrifugation at 10,000g for 15 min, and the radioactivity in the precipitates was determined. Samples were considered antibody-positive if counts were more than three times background levels.

Serum samples were also tested for antibody to p24 and p19 by a Western blot technique. Materials and the procedure are essentially as described by Tsang et al [40]. Details relevant to the present application are as follows: HTLV-I purified from an HTLV-I infected cell line (MT-2) [11,40] was suspended in .01 M Tris buffer, pH 8.0, containing 1% sodium dodecyl sulfate (SDS), 0.25 μg/ml bromphenol blue; 10% u/u glycerol, and 5% u/u 2-merceptoethanol, heated at 65°C for 30 min, and electrophoresed in a single well of a 3.3%–20% gradient polyacrylamide gel (PAGE; 20 mA/gel constant current, 10°C, until the blue marker reached the bottom of the gel). PAGE-resolved proteins were electrophoretically transferred to nitrocellulose sheets (60 V × 3 hr, 4°C). Washed sheets were cut into 3-mm strips. Individual strips were incubated with 1:100 dilutions of serum overnight at 4°C, washed, incubated for 2 hr at room temperature with a peroxidase-conjugated antihuman immunoglobulin reagent, and washed again. Color reactions were developed with 3,3′diaminobenzidine and $H_2O_2$ [40]. Banding patterns were compared with that of a known positive serum diluted 1:4000.

Immunologic testing using standard techniques was performed at the Centers for Disease Control (CDC) [41,42]. Lymphocyte subpopulations were quantitated by

indirect immunofluorescence on a fluorescence-activated cell sorter (FACS IV; Becton-Dickinson, Sunnyvale, CA), using commercially available monoclonal antibodies (OKT3 for T cells, OKT4 for T helper/inducer cells [$T_H$] and OKT8 for T suppressor/cytotoxic cells [$T_S$]; Ortho Diagnostics, Raritan, NJ) and a fluorescein-conjugated antimouse immunoglobulin (CDC) [42]. Immunoglobulins G, A, and M were quantitated by nephelometry (Baker Chemicals, Allentown, PA).

## RESULTS

All Hem A and nontransfused participants were negative for p24 antibody (Table I). Three Thal (6%) and one SCA (4%) participants were p24 antibody-positive. Three of these participants were also positive for antibodies to HTLV-I associated p19 and p61 antigens; one with Thal was positive for p24 antibody only. p24 antibody results using the Western blot technique were 100% concordant with those obtained from the RIP technique. The difference in rate of seropositivity for cellular and noncellular blood product recipients was not significant ($p = 15$, two-tailed Fisher's exact test [FET]). It was not possible to obtain additional participants from the New York City area; however, when these data were combined with additional data from our laboratory for hemophiliacs from other geographic areas [43] the difference in overall rate of seropositivity for chronic cellular vs noncellular blood product recipients was significant (4/75 vs 1/168, $p = .03$ FET).

None of the p24 antibody-positive participants was born in the Caribbean, nor had any travelled to Haiti, the West Indies, Japan, or Africa in the past 3 years. Information on blood product usage was obtained on 41 Hem A (median 419,500 units of factor VIII over the previous 3 years, range 16,470–1,444,260), 48 Thal, and 22 SCA patients. Blood-product usage by antibody-positive participants ranged from a minimum of 26 units of FPRC to a maximum of 60 units of FPRC per year (Table II). Usage by antibody-positive Thal was well within the range of usage by the antibody-negative participants; the antibody-positive SCA had relatively high usage compared to the antibody-negative ones. Immunologic studies were performed on most of the participants; the findings for the four p24 antibody-positive patients were well within the range of those of the antibody-negative participants (Table III).

## DISCUSSION

It has been suggested that a new retrovirus related to HTLV-I, ie, HTLV-III or lymphadenopathy-associated virus (LAV), is the etiologic agent of AIDS [44–47]. The occurrence of antibodies to HTLV-I–related membrane antigens in persons with

**TABLE I. Antibody Status to p24 Antigen by Assay Technique and by Participant Group**

| Participants | RIP assay | Western blot | Both assays |
|---|---|---|---|
| Hemophilia A | 0/42[a] | 0/48 | 0/41 |
| Thalassemia | 3/49 | 3/48 | 3/47 |
| Sickle cell anemia | 1/26 | 1/24 | 1/24 |
| Controls | 0/18 | 0/18 | 0/16 |

[a]Positive/total tested.

**TABLE II. Units of Frozen Red Blood Cells (FPRC) Used by p24-Antibody-Positive Participants and by Antibody-Negative Thal and SCA Groups,* by Year (New York Study)**

| Patients with | Units of FPRC | | | |
|---|---|---|---|---|
| | 1981 | 1982 | 1983 | Total |
| Thalassemia | | | | |
| Positive | | | | |
| Patient 1 | 28 | 34 | 26 | 88 |
| Patient 2 | 45 | 35 | 30 | 110 |
| Patient 3 | 41 | 42 | 50 | 133 |
| Negative | | | | |
| Median | 33 | 36 | 36 | 106 |
| Range | 0–56 | 4–55 | 3–60 | 10–170 |
| SCA | | | | |
| Positive | | | | |
| Patient 4 | 35 | 47 | 60 | 142 |
| Negative | | | | |
| Median | 7 | 13 | 16 | 38 |
| Range | 0–36 | 0–44 | 0–59 | 11–124 |

*Participants with thalassemia (Thal) or sickle cell anemia (SCA). Usage information was available for all 49 Thal and for 22 of 26 SCA.

**TABLE III. Immune System Findings for Individual p24-Antibody-Positive Participants and for Antibody-Negative Thal and SCA Groups (New York Study, 1983)**

| Group | Lymphocyte count (cells/mm$^3$) | % $T_H$ | % $T_S$ | H/S (mg/dl) | IgG (mg/dl) | IgA (mg/dl) | IgM (mg/dl) |
|---|---|---|---|---|---|---|---|
| Thalassemia | | | | | | | |
| Positive | | | | | | | |
| Patient 1 | 15,028 | 25 | 16 | 1.6 | 1,550 | 277 | 153 |
| Patient 2 | 9,750 | 23 | 6 | 3.8 | 1,510 | 760 | 87 |
| Patient 3 | 5,460 | 36 | 21 | 1.7 | 1,830 | 797 | 118 |
| Negative | | | | | | | |
| Median | 6,110 | 30 | 16 | 1.8 | 1,515 | 277 | 112 |
| Range | 1,736–17,298 | 10–56 | 3–31 | 0.6–4.0 | 565–3,820 | 75–1,140 | |
| n | 41 | 47 | 47 | 47 | 46 | 46 | 46 |
| Sickle cell anemia | | | | | | | |
| Positive | | | | | | | |
| Patient 4 | ND[a] | 27 | 14 | 1.9 | 1,680 | 317 | 148 |
| Negative | | | | | | | |
| Median | 4,350 | 37 | 15 | 2.1 | 1,590 | 238 | 117 |
| Range | 1,054–8,398 | 12–67 | 5–33 | 1.1–5.2 | 847–3,470 | 38–470 | 48–251 |
| n | 21 | 25 | 25 | 25 | 25 | 25 | 25 |

[a]ND, not determined.

hemophilia or with AIDS [18,19] might be due to crossreactive antibodies to HTLV-III/LAV, but, additionally, HTLV-I might itself be an infection for which persons at risk for AIDS, or with AIDS, are at risk [15–17,20]. Because HTLV-I can be associated with neoplasms [1–6,11–14], transmission of the virus via blood products in the United States would be of public health concern, totally aside from any relationship it might have to the AIDS agent [8,9,32–35].

Persons with hemophilia are at heightened risk for AIDS, with nine of the 56 persons with hemophilia-associated AIDS having no record of receiving any blood products other than factor concentrate in the 5 years preceding their AIDS diagnosis (personal communications from patients' physicians). Persons with thalassemia and sickle cell anemia are not at heightened risk for AIDS; in none of the 44 transfusion-associated (nonhemophiliac) AIDS cases reported to CDC as of February 8, 1984, had the patients received their blood products for therapy of either hematologic disorder.

Here we compared the HTLV-I p24 antibody status of persons with hemophilia (but asymptomatic for AIDS), thalassemia, and sickle cell anemia, and a geographically matched nontranfused control group and found that no Hem A or nontransfused participant had detectable antibody to HTLV-I p24 core antigen. Unlike the Hem A participants, three Thal and one SCA participants had detectable antibody to HTLV-I p24 antigen by both RIP and Western blot assay techniques. In a previous evaluation, we had found that the p24 antibody-negative Hem A participants had immune system abnormalities similar to those found in AIDS patients; the Thal and SCA groups did not [38].

Our results support recent findings that suggest that HTLV-I is transmissible through cellular blood products [36,37].* Further, these results suggest that freezing cellular blood products does not prevent HTLV-I transmission. Despite our negative findings for Hem A participants, we cannot be totally certain that HTLV-I is not transmissible in factor concentrate for three reasons. First, our results might not accurately represent exposure to virus, because HTLV-I antibodies might be predominantly directed against the virus's surface glycoproteins [49]. Second, we might not have detected a low prevalence of antibody in persons with hemophilia because our sample size was inadequate. This possibility, however, appears less likely in light of our results from other hemophiliac groups combined with these from New York City. Third, factor concentrate is produced from donations made throughout the country, whereas the majority of cellular blood products used by our participants came from donations made in New York. Thus if the prevalence of HTLV-I is higher in New York City blood donors than in those from other locations, these cellular blood products might be at higher risk for HTLV-I contamination than are the factor concentrates, a reasonable possibility if persons at risk for AIDS or with AIDS are also at risk for acquiring HTLV-I [15–20]. These reasons might be given to explain an absence of antibody in our hemophiliac population; however, they do not discount the positive antibody findings in our Thal and SCA groups. Furthermore, the difficulty experienced in experimental attempts to transmit HTLV-I without cell-to-cell

*These results are also consistent with the possibility of infection with HTLV-II; HTLV-I and -II have cross-reactive p24 core antigens. We refer to HTLV-I only because of the rareness of known infection with HTLV-II. The public health implications of infection with HTLV-II might be similar to those for infection with HTLV-I; both have been associated with neoplasms.

contact suggests that the differences between our blood-product recipients represents a real difference in risk of exposure to HTLV-I for persons receiving noncellular and cellular blood products [36,50].

The above serologic findings, the relative risk of acquiring AIDS for factor and cellular blood-product recipients, and the reported p24 antibody-negative status of hemophiliac AIDS patients [43] support the contention that, although the AIDS agent can be transmitted through factor concentrate (noncellular) as well as cellular blood products, HTLV-I is preferentially transmissible through cellular blood products. The latter is consistent with the finding that HTLV-I–negative leukocytes can be transformed in vitro by packed red cells, whole blood, and platelet concentrates but not by fresh frozen plasma, from HTLV-I–positive donors, reportedly due to leukocytes present in the cellular preparations [36]. HTLV-I is associated with hematologic neoplasms [1–6,11–14] and might represent an infection now endemic in some areas of the United States. We therefore suggest that, independent of the problem of AIDS, the transmissibility of HTLV-I via blood products is of potential public health concern in the United States. More extensive evaluation of HTLV-I prevalence within the United States, of the relative risks of transmissibility associated with various types of cellular blood products, and of the short- and long-term outcomes of HTLV-I exposure is therefore advisable.

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## REFERENCES

1. Blattner WA, Blayney DW, Robert-Guroff M, et al: Epidemiology of human T-cell leukemia/lymphoma virus. J Infect Dis 147:406–416, 1983.
2. Poiesz BJ, Ruscetti FW, Reitz MS, et al: Isolation of a new type C retrovirus (HTLV) in primary uncultured cells of a patient with Sezary T-cell leukaemia. Nature 294:268–271, 1981.
3. Akagi T, Ohtsuki Y, Takahashi K, et al: Detection of type C virus particles in short-term cultured adult T-cell leukemia cells. Lab Invest 47:406–408, 1982.
4. Hinuma Y, Gotoh Y-I, Sugamura K, et al: A retrovirus associated with human adult T-cell leukemia: In vitro activation. Gann 73:341–344, 1982.
5. Gallo RC, Blattner WA, Reitz MS, Ito Y: HTLV: The virus of adult T-cell leukaemia in Japan and elsewhere. Lancet 1:683, 1982.
6. Blayney DW, Jaffe ES, Blattner WA, et al: The human T-cell leukemia/lymphoma virus associated with American adult T-cell leukemia/lymphoma. Blood 62:401–405, 1983.
7. Popovic M, Sarin PS, Robert-Guroff M, et al: Isolation and transmission of human retrovirus (human T cell leukemia virus). Science 219:856–859, 1983.
8. Gotoh YI, Sugamura K, Hinuma Y: Healthy carriers of a human retrovirus, adult T-cell leukemia virus (ATLV): Demonstration by clonal culture of ATLV-carrying T cells from peripheral blood. Proc Natl Acad Sci USA 79:4780–4782, 1982.
9. Miyoshi I, Fujishita M, Taguchi H, et al: Caution against blood transfusion from donors seropositive to adult T-cell leukaemia-associated antigen. Lancet 1:683–684, 1982.
10. Kalyanaraman VS, Sarngadharan MG, Bunn PA, et al: Antibodies in human sera reactive against an internal structural protein of human T-cell lymphoma virus. Nature 294:271–273, 1981.

11. Kalyanaraman VS, Sarngadharan MG, Nakas Y, et al: Natural antibodies to the structural core protein (p24) of the human T cell leukemia (lymphoma) retrovirus found in sera of leukemia patients in Japan. Proc Natl Acad Sci USA 79:1653–1657, 1982.
12. Robert-Guroff M, Kalyanaraman VS, Blattner WA, et al: Evidence for human T cell lymphoma-leukemia virus infection of family members of human T cell leukemia lymphoma-virus positive T cell leukemia-lymphoma patients. J Exp Med 157:248–258, 1983.
13. Schupbach J, Kalyanaraman VS, Sarngadharan MG, et al: Antibodies against three purified proteins of the human type C retrovirus, human T-cell leukemia-lymphoma virus, in adult T-cell leukemia-lymphoma patients and healthy blacks from the Caribbean. Cancer Res 43:886–891, 1983.
14. Yoshida M, Miyoshi J, Hinuma Y: Isolation and characterization of retrovirus (ATLV) and its association with adult T-cell leukemia. Gann Monogr 28:229–237, 1982.
15. Gallo RC, Sarn PS, Gelmann EP, et al: Isolation of human T-cell leukemia virus in acquired immunodeficiency syndrome (AIDS). Science 220:865–867, 1983.
16. Miyoshi I, Kobayashi M, Yoshimoto S, et al: ATLV in Japanese patient with AIDS. Lancet 2:275, 1983.
17. Gelmann EP, Popovic M, Blayney D, et al: Proviral DNA of a retrovirus, human T-cell leukemia virus, in two patients with AIDS. Science 220:862–865, 1983.
18. Essex M, McLane MF, Lee TH, et al: Antibodies to cell membrane antigens associated with human T-cell leukemia virus in patients with AIDS. Science 220:860–862, 1983.
19. Essex M, McLane MF, Lee TH, et al: Antibodies to human T-cell leukemia virus membrane antigens (HTLV-MA) in hemophiliacs. Science 221:1061–1064, 1983.
20. Black PH, Levy EM: The human T-cell leukemia virus and AIDS. N Engl J Med 309:856, 1983.
21. Evatt BL, Ramsey RB, Lawrence DN, et al: Acquired immunodeficiency syndrome in hemophilia patients. Ann Intern Med 100:499–504, 1984.
22. U.S. Depart. of Health, Education and Welfare: "Study To Evaluate the Supply-Demand Relationships for AHF and PTC Through 1980." (DHEW Publ. #77-1274). Washington, DC: U.S. G.P.O.
23. deShazo RD, Andes WA, Nordberg J, et al: An immunologic evaluation of hemophiliac patients and their wives—Relationships to the acquired immunodeficiency syndrome. Ann Intern Med 99:159–164, 1983.
24. Lederman MM, Ratnoff OD, Scillian JJ, et al: Impaired cell-mediated immunity in patients with classic hemophilia. N Engl J Med 308:79–83, 1983.
25. Luban NLC, Kelleher JF Jr, Reaman GH: Altered distribution of T-lymphocyte subpopulations in children and adolescents with haemophilia. Lancet 1:503–505, 1983.
26. Menitove JE, Aster RH, Casper JT, et al: T-lymphocyte subpopulation in patients with classic hemophilia treated with cryoprecipitate and lyophilized concentrates. N Engl J Med 308:83–86, 1983.
27. Gill JC, Menitove JE, Wheeler D, et al: Generalized lymphadenopathy and T cell abnormalities in hemophilia A. J Pediatr 103:18–22, 1983.
28. Jones P, Proctor S, Dickinson A, George S: Altered immunology in haemophilia. Lancet 1:120–121, 1983.
29. Lechner K, Niessner H, Bettelheim P, et al: T-cell alterations in hemophiliacs treated with commercial clotting factor concentrates. Thromb Haemostas (Stuttgart) 50:552–556, 1983.
30. Weintrub PS, Koerper MA, Addiego JE Jr, et al: Immunologic abnormalities in patients with hemophilia A. J Pediatr 103:692–695, 1983.
31. Evatt BL, Stein SF, Francis DP, et al: Antibodies to human T cell leukaemia virus-associated membrane antigens in haemophiliacs: Evidence for infection before 1980. Lancet 2:698–701, 1983.
32. Hinuma Y. Association of a retrovirus (ATLV) with adult T-cell leukemia: review of serologic studies. Gann Monogr 28:211–218, 1982.
33. Fleming AF, Yamamoto N, Bhusnurmath SR, et al: Antibodies to ATLV (HTLV) in Nigerian blood donors and patients with chronic lymphatic leukaemia or lymphoma. Lancet 2:334–335, 1983.
34. Miyoshi I, Taguchi H, Fujishita M, et al: Asymptomatic type C virus carriers in the family of an adult T-cell leukemia patient. Gann 73:339–340, 1982.
35. Saxinger WC, Gallo RC: Possible risk to recipients of blood from donors carrying serum markers of human T-cell leukaemia virus. Lancet 1:1074, 1982.
36. Miyamoto K, Tomita N, Ishii A, et al: Transformation of ATLA-negative leukocytes by blood components from anti-ATLA-positive donors in vitro. Int J Cancer 33:721–725, 1984.
37. Okochi K, Sato H, Hinuma Y. A retrospective study on transmission of adult T-cell leukemia virus by blood transfusion: Seroconversion in recipients. Vox Sang 46:245–253, 1983.

38. Jason J, Hilgartner M, Holman RC, et al: Immune status of blood-product recipients. JAMA 253:1140–1145, 1985.
39. Kalyanaraman VS, Sarngadharan MG, Nakao Y, et al: Natural antibodies to the structural core protein (p24) of the human T-cell leukemia (lymphoma) retrovirus found in sera of leukemia patients in Japan. Proc Natl Acad Sci USA 79:1653–1657, 1982.
40. Tsang VCW, Peralta JM, Simons AR: Enzyme-linked immunoelectrotransfer blot techniques (EITB) for studying the specificities of antigens and antibodies separated by gel electrophoresis. Meth Enzymol 92:377–391, 1983.
41. Chan WC, Check IJ, Heffner LT, et al: Prolymphocytic leukemia of helper cell phenotype: report of a case and review of the scientific literature. Am J Clin Pathol 77:643–647, 1982.
42. Hoffman RA, Kung PC, Hansen WP, Goldstein G. Simple and rapid measurement of human T lymphocytes and their subclasses in peripheral blood. Proc Natl Acad Sci USA 77:643–647, 1980.
43. Chorba T, Jason J, Ramsey R, et al: Serosurveys for evidence of exposure to human T-Cell leukemia virus (HTLV-I) in European and American hemophilia patients treated with factor concentrates prepared from U.S. plasma sources and in hemophilia patients with acquired immunodeficiency syndrome. Thromb Haemostas, in press.
44. Barre-Sinoussi F, Chermann JC, Rey F, et al: Isolation of a T-lymphotropic retrovirus from a patient at risk for acquired immune deficiency syndrome (AIDS). Science 220:868–871, 1983.
45. Vilmer E, Barre-Sinoussi F, Rouzioux C, et al: Isolation of a new lymphotropic retrovirus from two siblings with haemophila B, one with AIDS. Lancet 2:753–757, 1984.
46. Popovic M, Sarngadharan MG, Read E, Gallo RC: Detection, isolation, and continuous production of cytopathic retroviruses (HTLV-III) from patients with AIDS and pre-AIDS. Science 224:497–500, 1984.
47. Sarngadharan MG, Popovic M, Bruch L, Schupbach J, Gallo RC: Antibodies reactive with human T-lymphotropic retroviruses (HTLV-III) in serum of patients with AIDS. Science 224:506–508, 1984.
48. Yamamoto N, Schneider J, Koyanagi Y, et al: Adult T-cell leukemia (ATL) virus-specific antibodies in ATL patients and healthy virus carriers. Int J Cancer 32:281–287, 1983.
49. Miyoshi I, Taguchi H, Kubonishi I, et al: Type C virus-producing cell lines derived from adult T-cell leukemia. In Hanaoka M, Takatsuki K, Shimoyama M (eds): "Adult T-Cell Leukemia and Related Diseases." New York: Plenum Press, 1982, pp 219–228.
50. Yamamoto N, Okada M, Koyanagi Y, et al: Transformation of human leukocytes by cocultivation with an adult T cell leukemia virus producer cell line. Science 217:737–739, 1982.