In vitro photochemical inactivation of cell-associated human T-cell leukemia virus Type I and II in human platelet concentrates and plasma by use of amotosalen

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BACKGROUND: Human T-cell leukemia virus Types I and II (HTLV-I and HTLV-II), blood-borne retroviruses found worldwide, can cause leukemia, immunosuppression, and severe neurologic diseases. In most countries, HTLV-I and -II screening is not performed systematically for blood donations. A new photochemical treatment (PCT) with a synthetic psoralen was developed to inactivate most pathogens in platelet (PLT) concentrates or plasma and to improve the safety of blood donations.
STUDY DESIGN AND METHODS: Cell-associated HTLV-I or -II (10^6 /mL) was inoculated in full-size fresh PLT concentrates or fresh frozen plasma and treated with 150 μmol per L amotosalen (S-59) and different doses of long-wavelength ultraviolet A (UVA) light. The residual viral titer in the treated samples was assessed by a cocultivation assay on indicator cells.
RESULTS: The inactivation obtained at a 3.0 J per cm^2 UVA dose was greater than 5.2 log foci-forming units (FFUs) per mL for HTLV-I and 4.6 log FFUs per mL for HTLV-II in presence of human PLT concentrates and greater than 4.5 log FFUs per mL for HTLV-I and 5.7 log FFUs per mL for HTLV-II in the presence of human plasma. The residual infectivity was very low and shown as the limit of detection of the cocultivation assay.
CONCLUSION: In human plasma or PLT concentrates, the retroviruses HTLV-I and -II were strongly sensitive to the PCT with 150 μmol per L amotosalen (S-59) and a 3.0 J per cm^2 UVA dose. This high efficiency for photoinactivation of these retroviruses opens a possibility of improving the safety of PLTs or plasma transfusion in the future.

The human T-cell leukemia virus Type I (HTLV-I) is the first pathogenic agent belonging to the retroviridae family discovered.[1] HTLV-I provided proof of a relationship between viruses and cancer and it is known to be the etiologic agent of adult T-cell leukemia (ATL), an aggressive CD4+ T-cell malignancy[2,3] and of HTLV-I–associated myelopathy, also known as tropical spastic paraparesis (TSP).[4,5] TSP is a chronic and progressive neuromyelopathy that leads to severe handicaps, frequently after a long latency period. No treatment of HTLV-I–associated lymphoproliferative malignancies has been reported to reduce mortality of patients with ATL and the course is usually fatal. No specific treatment for the myelopathy is known. HTLV-I infection is also frequently encountered in other syndromes, including ophthalmologic complications,[6] infectious dermatitis,[7] alveolar pneumopathies,[8] arthropathies,[9] polymyositis,[10] uveitis,[11] and immunodepression.[12] There is evidence that dual infection with HTLV-I and human immunodeficiency virus (HIV) accelerates the progression of the immunodeficiency syndrome (AIDS).[13,14] The risk of developing an

ABBREVIATIONS: ATL = adult T-cell leukemia; CA = cell-associated (HTLV-I); FFU(s) = foci-forming unit(s); PAS = platelet additive solution; PCT = photochemical treatment; PMA = 12-O-tetradecanoylphorbol-13-acetate; TSP = tropical spastic paraparesis; UVA = ultraviolet A (320- to 400-nm light).

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HTLV-I–associated disease such as ATL and TSP is approximately 5 percent in asymptomatic infected persons. A closely related retrovirus, human T-cell leukemia virus Type II (HTLV-II), was isolated from a patient with hairy-cell leukemia in 1982,[15] and its pathogenicity remains unclear despite its association with a few cases of neuromyelopathy and lymphoproliferative disorders.[16-18] Recently, HTLV-II was shown to increase the risk of developing peripheral neuropathy in patients coinfected with HIV-1[19,20] and seems to be a cofactor in AIDS progression.[21]

HTLV-I is found worldwide but is not ubiquitous, with infection rates ranging from 0 to 50 percent in some clusters.[22] Areas of HTLV-I endemicity include Japan,[23,24] west and tropical Africa,[25] the Caribbean Islands,[26] some regions in north and south America,[27,28] and Melanesia.[29] HTLV-II endemically infects some American Indian and African populations,[30-34] and the infection is increasing in intravenous (IV) drug users in developed countries, including the United States,[35] Europe,[36] and Asia.[37] A total of 15 to 25 million people are estimated to be infected by HTLV-I and/or -II. The major modes of transmission are perinatal (mainly through breastfeeding),[38] parenteral (through blood transfusions or exposure to needles and syringes contaminated with blood),[39] and sexual.[40]

Blood transfusion is a very efficient mode of transmission of HTLV-I and -II.[41-43] The virus is transmitted mainly by T-lymphocytes, although infection with cell-free virus may occur with less efficiency. Plasma and platelet (PLT) preparations are usually HTLV-I and -II–free, but recently, the persistence of HTLV-I in blood components after whit blood cell (WBC) depletion was demonstrated.[44] Acquiring the virus by transfusion increases the risk of developing ATL and TSP, as well as of increasing the severity of symptoms.[45-47] Prevention of HTLV-I infection includes systematic screening of blood transfusions,[48,49] avoidance of breastfeeding, protected sex, and vaccine development. But the efficiency of these methods is limited and a vaccine against HTLV-I and -II is not yet available. Moreover, screening has not been implemented in every country in which infections have been documented, and the risk of transfusion-associated transmission is closely associated with the long asymptomatic phase following the primary infection, during which the individuals are unknowingly contagious. Thus, blood transfusion still carries a significant risk for HTLV-I and -II infection in endemic areas.

To improve the safety of blood component transfusion, a new strategy of decontamination of blood products has been developed. This new photochemical treatment (PCT) process uses the Helinx technology (Cerus Corp., Concord, CA) to inactivate potential pathogens. It utilizes a synthetic psoralen (amotosalen or S-59) and long-wavelength ultraviolet A (UVA) illumination.[50] Amotosalen is a nucleic acid–targeting reagent which, upon UVA illumination, forms covalent adducts with nucleic acid and prevents replication, rendering free or cell-associated pathogens noninfectious. This treatment has already been shown to inactivate high levels of a large spectrum of viruses (free or cell-associated and proviral DNA), bacteria, protozoans, and WBCs.[51-54] Among retroviruses, HIV inactivation was demontrated.[55] In contrast, PLTs and plasma do not require nucleic acid for therapeutic function and are not greatly affected by PCT.[56,57] Amotosalen is used at a final concentration of 150 µmol per L with a 3.0 J per cm^2 UVA illumination dose for a PLT unit containing approximately 4.0×10^{11} PLTs per 300 mL or for a plasma unit corresponding to a volume of 600 mL. To fully assess this technology, systems allowing the evaluation of the efficiency of pathogen inactivation were necessary. Astier-Gin and colleagues[58] have developed an in vitro coculture assay that allows to titrate cell-free and cell-associated HTLV-I and -II viruses. We have used this system to demonstrate the inactivation of HTLV-I and HTLV-II with HTLV-I or HTLV-II chronically infected cells spiked into PLT concentrates or plasma. The study included four separate experiments for each type of virus with different fresh human PLT concentrate units or human fresh frozen plasma (FFP) units.

MATERIALS AND METHODS

PCT of HTLV-I and -II in fresh human PLT concentrates

PLT concentrates. The four separate inactivation assays of the cell-associated HTLV-I and -II in presence of PLTs were performed in different full-size donor PLT concentrate units. Approximately 4.0×10^{11} fresh single-donor PLTs were collected the day before each experiment (French Blood Establishment/EFS, Bordeaux, France) on a cell separator (Amicus, Baxter Healthcare Corp., Belgium) resuspended in approximately 105 mL of autologous plasma and approximately 180 mL of PLT additive solution (PAS III; Baxter Healthcare Corp.) in a 1-L PL2410 container and shipped the day of the experiment. Serologic tests for detection of HTLV-I and -II antibodies were performed before delivery for the experiments. The PLTs were diluted with extra autologous plasma. The PLT concentrate units were weighted and adjusted with 35 percent autologous plasma and 65 percent PAS III to obtain 285 mL as final volume.

Cell-associated HTLV-I and -II preparation. The 2060 HTLV-I–infected lymphoid cell line, established from a TSP patient from French Guyana,[59] or C19 HTLV-II–infected cell line, established from peripheral blood mononuclear cells of an IV drug user from California,[60] were cultured in RPMI 1640 (Cambrex Corp., East Rutherford, NJ; Moore et al., Roswell Park Memorial Institute) supplemented with 10 percent heat-inactivated fetal calf serum (Cambrex Corp.), 100 U per mL penicillin, 0.1 mg per mL streptomycin (Sigma Chemical Co., St. Louis, MO),

and 200 U per mL interleukin-2 (Sigma Chemical Co.) for the 2060 line. HTLV-I–infected cells (2060) or HTLV-II–infected cells (C19) were cultured in flasks, pooled, and counted, to obtain a final concentration of 10^6 cells per mL in each PLT unit. The culture was centrifuged to pellet approximately 3.0×10^8 cells. The pelleted cells were resuspended in 31 mL of 35 percent plasma and 65 percent PAS III. One milliliter of this suspension was used for the control titration of the inoculum.

PLT unit preparation. Pooled HTLV-I– and -II–infected cells (30 mL) were added to the PLT concentrate (285 mL), resulting in approximately a 1:10 dilution of infected cells into PLTs (approx. 10^6 cells/mL final concentration). HTLV-I– and -II–infected cells and PLTs were thoroughly mixed up, and 30 mL of the suspension was removed, from which a 28.5-mL aliquot (10% of the test volume) was added to an empty plastic container designated as control. The resulting large unit containing 285 mL of HTLV-PLTs suspension was designed as test.

Photochemical inactivation. Fifteen milliliters of amotosalen (3 mmol/L in saline solution) was added to the test unit and immediately transferred into a 1 L plastic illumination container (Baxter Healthcare Corp.), which ensured adequate mixing. Saline solution (1.5 mL 0.9%) was added into the control unit containing 28.5 mL of HTLV-PLTs suspension to bring the volume to 30 mL. After mixing, a 1 mL aliquot was withdrawn from the test unit for measurement of the initial titer (sample test at 0 J/cm^2). The test unit was then illuminated on a UV illumination system (FX1019, Modified 4R4440; Baxter Healthcare Corp.) for the prescribed number of J per cm^2. Four samples were removed successively, after exposition to 0.5, 1.0, 2.0, and 3.0 J per cm^2 UVA doses, respectively, to measure the titer of residual infectivity (1 mL for 0.5-2 J/cm^2 and 10 mL for 3 J/cm^2). In parallel, 1-mL aliquots were removed from the control unit (samples concurrent with the 0, 0.5, 1.0, 2.0, and 3.0 J/cm^2 test samples).

Titration with indicator cell cocultivation assay. For the first test sample (not treated) and the control samples, 10-fold serial dilutions were made, and two 2-mL aliquots were plated on indicator cells for each of the four dilutions performed. For the four inactivated samples, 5 (0.5-2.0 J/cm^2) or 45 (3.0 J/cm^2) 2-mL aliquots were plated. To avoid toxicity to the pA18G-BHK-21 cells, the medium of plated samples was changed in each well after 2 hours of coculture, allowing the sedimentation of the infected cells that were not removed during the change. The titration of the HTLV-I and -II infectivity in every test and control sample was evaluated with an in vitro system described by Astier-Gin and coworkers[57] including the modification appropriate for analysis of samples containing PLTs or plasma to avoid cell clotting (15 U/mL heparin sulfate). The system is based on the identification of HTLV-I– and HTLV-II–producing cells by cocultivation with pA18G-BHK-21 indicators cells. These cells are stably transfected with a LTR_{HTLV}-lacZ gene construct that is transactivated by the tax activator protein of the HTLV-I and -II and allows the production of β-galactosidase. pA18G-BHK-21 indicators cells appear in blue in presence of 5-bromo-4-chloro-3-indolyl β-D-galactopyranoside substrate (X-Gal; Sigma Chemical Co.). The number of positive colored cells, focus-forming units (FFUs), is directly proportional to the number of HTLV-I– and -II–infected cells existing in the plated aliquots. pA18G-BHK-21 cells were seeded in 24-well plates the day before each experiment and incubated for 24 hours to initiate an indicator cell monolayer. The test and control sample dilutions were plated on the pA18G-BHK-21 monolayer and after 72 hours of coculture with 10^{-7} mol per L 12-*O*-tetradecanoylphorbol-13-acetate (PMA; Sigma Chemical Co.) to enhance HTLV production, the β-galactosidase expression was measured. The confluent adhesive indicator cells were washed with phosphate-buffered saline and fixed by 1 mL of 0.2 percent glutaraldehyde-1 percent formaldehyde mix, and X-Gal was added to each well and incubated 4 hours at 37°C to allow the formation of colored product inside infected indicator cells. The number of blue cells was counted with a microscope (Axiovert 200, A-plan 5×/0.12 objective, Zeiss, Germany). Wells containing only pA18G-BHK-21 in RPMI 1640 were used as negative control to obtain a background value. The titer was determined by linear regression for each sample, and the inactivation log was calculated (Fig. 1). The experiments were performed four

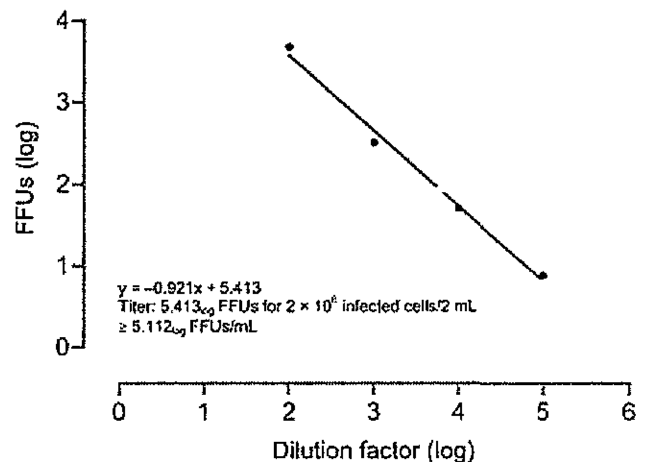

Fig. 1. Typical linear regression for titration of HTLV-I and -II infectivity: With the correlation between the mean level of FFUs counted and the corresponding dilution, a linear regression allowed us to determine the HTLV-I and -II infectious titer in the original samples from blood component units. Data shown are from the first replicate of HTLV-II inactivation in PLT concentrates: sample withdrawn in control unit, with four dilutions (log 2 to log 5) assayed. The linear regression allowed us to estimate the original titer at 5.112 log FFUs per mL (p = 0.004).

times but a fifth replicate assay was performed for HTLV-II (see Results) and the results were averaged.

PCT of HTLV-I and -II in fresh plasma

Plasma concentrates. Fresh single-donor plasmas were collected (Sacramento Blood Center, Sacramento, CA) in 600-mL plastic containers before being frozen and delivered for experimentation. The day of experimentation, the plasma was thawed in a 37°C water bath for 1 hour and left at room temperature for 1 additional hour.

Cell-associated HTLV-I and -II preparation. HTLV-I–infected cells (2060) or HTLV-II–infected cells (C19) were cultured in flasks, pooled, and counted, to obtain 10^6 cells per mL final concentration in the plasma units. The required volume of culture was centrifuged to pellet approximately 1.2×10^9 cells. The pelleted cells were resuspended in plasma to a final volume of approximately 32 mL. A 0.1-mL aliquot of this suspension was reserved for titration of the inoculum.

Plasma unit preparation. Three plasma units were pooled in a transfer container. The total volume of the plasma pool was determined by weight and adjusted to 1200 mL. Heparin was then added (3.6 mL; 15 U/mL final concentration). The cell suspension (approx. 30 mL) was added into the transfer container resulting in approximately 10^6 infected cells per mL final concentration. After thorough mixing, the suspension was split into two 585-mL units (Test 1 and Test 2).

Photochemical inactivation. Fifteen milliliters of amotosalen (6 mmol/L in saline solution) was added to the test plasma and immediately transferred into a 1-L plastic illumination container, which ensured adequate mixing. After mixing, an aliquot was withdrawn for measurement of the initial titer (Test 1 and Test 2 at 0 J/cm^2). The test units were then illuminated on an UV illumination system (FTC 0054, 4R4008, Baxter Healthcare Corp.) for the prescribed number of J per cm^2. Samples were removed after a single 1.0 and cumulative 2.0 J per cm^2 UVA dose for Test 2 and a single 3.0 J per cm^2 UVA dose in Test 1 for measurement of the titer. Sampling from 2 separate units originating from the same plasma pool, which allowed us to consider the results for the four UVA doses as only one kinetic curve of inactivation.

Titration with indicator cell cocultivation assay. For 0 J per cm^2 samples from Test 1 and Test 2, 10-fold serial dilutions were made, and two 2-mL aliquots were plated for each of the four dilutions performed. For the inactivated samples from Test 2 (1 or 2.0 J/cm^2) or Test 1 (3.0 J/cm^2), 45 2-mL aliquots were plated. The medium of the plated samples was changed after 2 hours of coculture for the same reason as in the experiments in the presence of PLTs. The titration of the HTLV-I and -II infectivity in every test sample was evaluated with the same method as described previously for PLTs. Negative controls were performed both in RPMI 1640 only and in RPMI 1640 containing 10 percent plasma (from pooled plasma samples before addition of infectious cells). The experiments were performed four times for each virus type, and the results were averaged.

RESULTS

The control samples (PLT experiments) or test samples before illumination (plasma experiments) allowed us to obtain a referential infectious titer (FFUs/mL) by linear regression. The residual foci detected in the inactivated samples and the negative controls were counted and the mean was calculated. Dose-response curves were generated to visualize the efficiency of the inactivation. It is important to emphasize that most of the FFUs correspond either to one colored cell before multiplication and formation of a focus or sometimes to a syncytia. The wells containing negative controls gave a number of infectious foci by well (2 mL), which corresponds to the residual infectivity or the limit of detection and, thus, was evaluated to a value in FFUs per mL.

PCT of HTLV-I and -II in fresh human PLT concentrates

Photochemical inactivation of HTLV-I. The cell-associated HTLV-I showed a strong sensitivity to amotosalen with a low dose of UVA. The inactivation curve dropped rapidly in the four replicates (Fig. 2A): in the first sample treated by 150 μmol per L amotosalen and a 0.5 J per cm^2 UVA dose, residual infectivity was less than 0.1 percent. From the 1.0 J per cm^2 UVA dose, the value of the infectivity reached a background value equal to the negative control obtained in RPMI 1640 alone. The mean value obtained for the 3.0 J per cm^2 UVA dose inactivated sample was -0.5 ± 0.2 log FFUs per mL. In contrast, the titer obtained in the control samples was stable and gave a mean of 4.7 ± 0.2 log FFUs per mL. So, the strong inactivation of 5.1 log by a 1.0 J per cm^2 UVA dose allowed us to conclude that the free and cell-associated HTLV-I inactivation by 150 μmol per L amotosalen and 3.0 J per cm^2 UVA was complete with respect to the limit of detection of the cocultivation assay used.

Photochemical inactivation of HTLV-II. Like for HTLV-I, in the four replicates a strong inactivation from the first UVA dose was observed (Fig. 2B). In the first sample treated by 150 μmol per L amotosalen and a 0.5 J per cm^2 UVA dose, a residual infectivity decreased strongly. After a 1.0 J per cm^2 UVA dose, the value of the infectious titer reached a background value slightly higher than the value obtained in the negative control in RPMI 1640 alone. The mean value obtained for the most illuminated sample (3.0 J/cm^2) was 0.4 ± 0.3 log FFUs per mL. In contrast, the titers obtained in the control samples were stable and

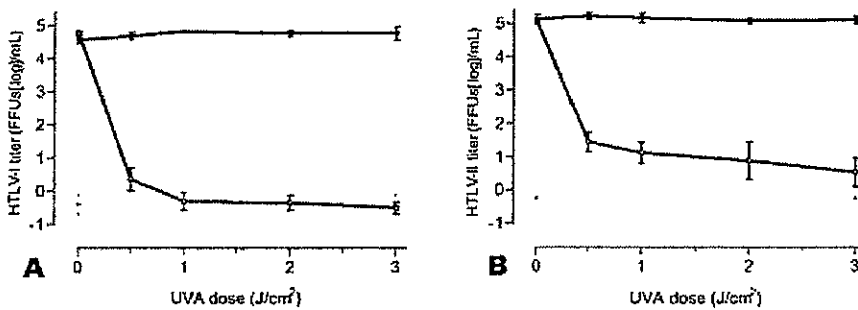

Fig. 2. Dose-response curves of the photochemical inactivation of HTLV-I (A) and HTLV-II (B) in PLT concentrates (n = 4). The inactivation of CA-HTLV-I and -II in PLTs by PCT was performed in four separate replicates, and results were pooled for each UVA dose. Error bar represents the standard deviation (SD) from the mean. The negative control (solid gray line) was given by FFU count in RPMI 1640 only. Stable for all UVA doses, it was expressed as a single mean value represented with its SD (double dashed gray lines). (●) Infectious titer obtained in a control PLT unit containing HTLV-I or -II without PCT. These samples were withdrawn in parallel with the TEST ones. (○) Infectious titer obtained in test PLT unit containing HTLV-I or -II with PCT at each UVA doses.

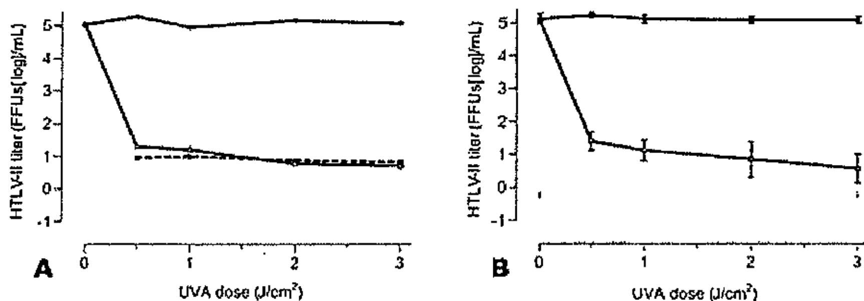

Fig. 3. Dose-response curve of the additional replicate of photochemical inactivation of HTLV-II in PLT concentrate units (A) and new application of this limit of detection to the previous photochemical inactivations of HTLV-II in PLT concentrates (B; n = 5). (A) The fifth inactivation of CA-HTLV-II in PLTs by PCT was in two separate PLT units with or without HTLV-II. The classical negative controls (without treatment) were given by counting of FFUs in RPMI 1640 only (solid gray line) or in RPMI 1640 added of 10 percent plasma (dashed gray line). The additional limit of detection was measured in the second PLT unit without HTLV-II but treated by UVA and amotosalen 150 μmol per L. (■) Limit of detection of the pA18G-BHK-21 cocultivation assay determined in presence of PLTs/plasma after PCT. (●) Infectious titer obtained in control PLT unit containing HTLV-II without PCT. (○) Infectious titer obtained in test PLT units containing HTLV-II with PCT. (B) Curve comparing the new limit of detection to the four previous photochemical experiments in PLTs and confirming inactivation of HTLV-II in PLTs to the limit of detection.

gave a mean from 5.0 log to 5.2 log FFUs per mL. So the inactivation after a 3.0 J per cm² UVA dose was 4.6 log but the curve decreased to a background value slightly higher than that of the negative control in RPMI 1640.

To understand better the reason why a residual infectivity persisted in the inactivated samples in HTLV-II experiments, we tested the impact of the several components of the cocultivation assay on pA18G-BHK21 indicator cells in presence of PLTs and plasma. Therefore, to assess the capacity of the assay to generate a positive reaction under the conditions used in these experiments, excluding the real infectious power of residual HTLV-II virus, a fifth replicate HTLV-II experiment was performed as a double assay. This experiment was performed under exactly the same conditions as the previous four replicates but the usual PLT unit was used in parallel with another identical PLT unit in which no infectious agent was added and both were treated by UVA and amotosalen. The results of this additional experiment (Fig. 3A) showed an HTLV-II inactivation profile identical to the previous replicates: the curves dropped rapidly at 0.5 J per cm² and decreased to a background value at 2.0 J per cm² but stayed above the negative control in RPMI 1640. The additional unit, however, brought some new information: the number of blue cells appearing on the pA18G-BHK-21 indicator cells monolayer in the negative control without HTLV-II was higher in presence of PLTs than in RPMI 1640 alone. Moreover, in the samples receiving 3.0 J per cm², the titer was identical irrespective of the presence of HTLV-II. Because there was no difference between the samples irradiated by different dose of UVA and the negative control in 10 percent plasma (Fig. 3A), the UVA dose or the amotosalen seemed to have no impact on the background value. The theory of an abnormal sensitivity of the indicator cells monolayer to the psoralen or the UVA dose can be discarded. Because the experiments with HTLV-II have been realized some months after those with HTLV-I by use of the same pA18G-BHK-21 cell culture, cell aging or an evolution of the sensitivity could explain this changes. Alternatively, a change in plasma and PLT quality can be hypothesized. We can conclude that the inactivated samples reached a background value that was equivalent to the limit of detection of the cocultivation assay on pA18G-BHK-21 indicator cells (negative controls) in presence of PLTs and autologous plasma. Presumably, an extrapolation of this

result would be possible for the other replicates where negative controls in plasma had not been made. Therefore, we added the new limit of detection in presence of PLTs to the previous results by taking the mean of the 5 replicates (Fig. 3-B). So, we obtained a curve that reached the background value corresponding to zero infectious agents in the sample tested after a UVA dose of 1.0 J per cm^2.

PCT of HTLV-I and -II in human FFP

Photochemical inactivation of HTLV-I. The titrations of samples containing 150 µmol per L amotosalen before illumination (0 J/cm^2) gave a mean initial titer of 4.0 log FFUs per mL in Test 1 and Test 2. The inactivated samples also showed a low number of residual foci at −0.5 log FFUs per mL, a value equivalent to the background value obtained in negative controls of RPMI 1640 with 10 percent plasma (Fig. 4).

So the inactivation calculated for both test units at every UVA dose was 4.7 ± 0.3 log, and the residual infectivity observed corresponded to the limit of detection of the assay. Therefore, the inactivation of cell-associated (CA)-HTLV-I in a human plasma unit, by 150 µmol per L amotosalen plus a 3.0 J per cm^2 UVA dose, was complete at the limit of detection.

Photochemical inactivation of HTLV-II. The same observation can be applied to the results of the PCT of HTLV-II in plasma. The initial titer was very close in Test 1 and Test 2: 4.7 ± 0.1 log and 4.7 ± 0.3 log FFUs per mL, respectively, at 0 J per cm^2. The residual foci detected in inactivated samples gave −0.5 ± 0.8 log FFUs per mL for the 1.0 J per cm^2 UVA dose and −1 log FFUs per mL for 2.0 or 3.0 J per cm^2 UVA doses. Again, these residual foci corresponded to the background value, indicating that the inactivation of PCT on CA-HTLV-II in human plasma was very efficient. (Fig. 4). The corresponding inactivation log at a 3.0 J per cm^2 dose was 5.7 ± 0.1 log.

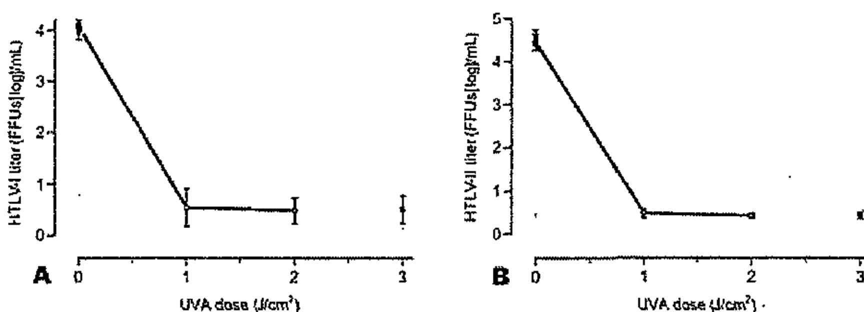

Fig. 4. Dose-response curves of the photochemical inactivation of HTLV-I (A) and II (B) in plasma (n = 4). The inactivation of CA-HTLV-I and -II in plasma by PCT was performed in four separate replicates, and results were pooled for each UVA dose. Each error bar represents the SD from the mean. The negative control (solid gray line) was given by FFU count in RPMI 1640 added of 10 percent plasma: Stable for all UVA doses it was expressed as a single mean and represented with its SD (double dashed gray lines). (●) Infectious titer obtained in Test 1 plasma unit containing HTLV-I and -II with PCT at 0 and 3.0 J per cm^2 UVA dose. (○) Infectious titer obtained in Test 2 plasma unit containing HTLV-I or -II with PCT at 1.0 and 2.0 J per cm^2 UVA dose.

DISCUSSION

The pA18G-BHK-21 cells transfected with the HTLV-LTR-lacZ and used for the coculture assay developed by Astier-Gin and coworkers[58] are indicator cells sensitive to the presence of tax activator protein produced by HTLV-I and -II. They also express HTLV-I and -II receptors that realize a possible recognition and fusion via the specific glycoprotein of the virion (infection) or of the infected cell membrane (syncytia). The main ways of expression of the tax protein in indicator cells is either an infection by direct penetration of the virion in the indicator cell or the fusion of the indicator cell with infected cells (some syncytia were including in the count of the positive FFUs in the stained indicator cell monolayer). Therefore, the cocultivation assay allowed us to measure the presence of free-cell, intracellular, or proviral forms of the HTLV-I and -II.

Lin and associates[61] have previously reported that PMA induced HTLV-LTR gene expression and therefore strongly increased tax protein expression (three to four-fold as determined by densitometric analysis of Western blot). HTLV-I and -II virions themselves, however, or cell-free HTLV-I and -II, have rarely been used to elucidate the infection mechanism. In contrast to HIV, cell-free viremia in plasma is not a prominent aspect of HTLV-I– and -II–associated diseases and the studies have focused on the proviral load in peripheral blood mononuclear cells. It is now admitted, however, that infection by cell-free HTLV-I and -II virions is less efficient compared with that of other retroviruses. The proportion of free viruses in culture supernatant in vivo has never been clearly estimated.[62] Astier-Gin and associates[58] confirmed that there was extremely little activation of the HTLV-LTR contained in pA18G-BHK-21 by HTLV-I and -II cell-free viral preparation in contrast to infected cells preparation. In contrast, even if free particles in the culture are not quantified, their presence is real and, if so, increased by the addition of PMA. By taking into account this information, the assays was designed to assess the inactivation of both free and cell-associated HTLV-I and -II without knowing their respective proportion.

Moreover, the virus present in the transfusions samples represents mostly a latent infection (proviral DNA). This does not exclude the pres-

ence of free virus potentially produced by the infected cell. The 2060- and C19-infected cell lines used in this work contained proviral HTLV-I and -II and produced virus particles. This production was increased in presence of PMA. Thus, the inactivation of virus produced by these cells was made with higher cell concentration (10^6 infected cells/mL) than those found in the transfused blood units, demonstrating with reliability that every virus form (free, intracellular, or proviral) was killed. That confirms that the target of the treatment by amotosalen was nucleic acids and that every form of replicating or latent viral gene was inactivated.

Bringing the proof that a strong concentration of HTLV-I– and -II–infected cells (probably containing the virus under its three forms) is destroyed in our experiments, we could expect that whatever the form and the concentration of HTLV-I and -II in transfused blood unit, the efficiency of the amotosalen and UVA will be equivalent. These results are concordant and demonstrated a similar inactivation profile: an important decrease of the infectivity power with a 1.0 J per cm^2 UVA dose, with a residual very low infectivity, reaching the limit of detection at a 3.0 J per cm^2 UVA dose. Moreover, they showed an inactivation of 5.2 log for HTLV-I or 4.6 log for HTLV-II in fresh human PLT concentrates and an inactivation of 4.5 or 5.7 log, respectively, in human plasma. The samples tested without treatment by UVA and amotosalen or before UVA illumination showed stability of the initial infectivity in the blood component used and the absence of inhibition of the HTLV-I– and -II–infected cells by the presence of PLT concentrate or plasma. The background value reached for the HTLV-II inactivation in presence of PLT concentrates, however, was slightly higher than that of the negative control in RPMI 1640 only. This could raise some questions about the efficiency of the PCT and the possibility of a residual infectivity. Nevertheless, a fifth replicate assay with PLTs, tested with or without HTLV-II, has demonstrated the susceptibility of the indicator cells (pA18G-BHK-21) to the presence of PLTs or the autologous plasma, which increased the limit of detection in contrast to the negative control in RPMI 1640 only. Actually, even if the PLTs quantity remained low when the sample dilutions were plated on the pA18G-BHK-21 monolayer (1 log dilution for inactivated samples with medium change after 2 hr), the contact nevertheless does exist. In addition, aging of the pA18G-BHK-21 cells or a change in the plasma components used should not be disregarded as the cause of the slightly higher background counts.

Taking into account the equivalence between the residuals titer in inactivated samples and the value of the limit of detection of the cocultivation assay on pA18G-BHK-21 indicator cells in presence of PLTs or plasma, and the high inactivation log obtained (Table 1), we can consider that the inactivation of cell-associated HTLV-I or HTLV-II in human plasma or in fresh PLT concentrates, by the PCT applied in these experiments (150 µmol/L amotosalen and an illumination dose of 3.0 J/cm^2), was highly efficient and opens an opportunity to improve safety of PLTs or plasma transfusions in the future.

TABLE 1. Inactivation of HTLV-I and -II in PLTs or plasma by 150 µmol per L amotosalen and different UVA doses (n = 4)*

UVA dose (J/cm^2)	Inactivation in PLTs (log)†		Inactivation in plasma (log)‡	
	HTLV-I	HTLV-II	HTLV-I	HTLV-II
0.5	4.3	3.8	ND	ND
1.0	5.1	4.0	4.6	5.2
2.0	5.0	4.3	5.1	5.6
3.0	5.2	4.6	4.5	5.7

* Mean values of the four replicates realized for each virus type.
† The inactivation log was calculated as the ratio of the mean titer (FFUs/mL) in the 0 J/cm^2 samples from the control units containing 150 µmol per L amotosalen to the mean titer (assimilated to FFUs/mL) in the irradiated samples from the test unit. (Control and test samples being concurrently withdrawn after each UVA dose.)
‡ The inactivation log was calculated as the ratio of the mean titer (FFUs/mL) in the 0 J per cm^2 samples (Test 1 or 2 unit containing 150 µmol per L amotosalen before UVA irradiation) to the mean titer (assimilated to FFUs/mL) in the irradiated samples.

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