UVB Irradiation of Human Platelet Concentrates Does Not Prevent HLA Alloimmunization in Recipients

By M.A. Grijzenhout, M.I. Aarts-Riemen, F.R. de Gruyl, H. van Weelden, and H.C. van Prooijen

Exposure of platelet concentrates (PCs) to ultraviolet B radiation (UVB) has been advocated as an alternative method for prevention of the onset of HLA sensitization in recipients. In this study, pooled PCs were irradiated in a Haemonetics UV irradiator (Haemonetics Corp, Braintree, MA) at a dose that did not induce platelet activation. The effect of UVB irradiation on prevention of primary HLA sensitization was evaluated in a prospective controlled clinical study performed in cardiac patients undergoing cardiopulmonary bypass. Patients were treated with filtered red blood cells and a single transfusion of either standard (control group) or UVB-irradiated (UVB group) pooled platelets prepared from 12 donors. Five of 39 patients in the control group and 6 of 62 patients in the UVB group developed allo-antibodies against HLA antigens, which is not significantly different (P = .62). This unexpected finding prompted us to check the efficacy of UVB irradiation. We determined UVB-specific DNA damage in cells by measuring the fluorescence from a labeled specific monoclonal antibody against thymine dimers. With this novel flow cytometer technique, we estimated that the mean fluorescence intensity (MFI) of 47 ± 2 arbitrary units (n = 6) correlated with abolition of alloreactivity in mixed lymphocyte cultures and delayed cell death (within 72 hours). MFI in leukocytes suspended in plasma and exposed to the clinical dose of UVB was sixfold higher (310 ± 41 arbitrary units) and resulted in early cell death (within 24 hours). We hypothesize that this high level of UVB radiation induces fragmentation of the leukocytes. As a consequence, the poor results of UVB irradiation may be explained by the onset of HLA-alloimmunization induced by soluble donor HLA class I antigens processed and presented by host antigen-presenting cells.

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Material and Methods

Determination of the Intermediate Dose of UVB

Pooled PCs were exposed to UVB in a commercially available UV irradiator (Platelet Treatment System; Haemonetics, Braintree, MA). For determining the intermediate dose of UVB, the effect of UVB on platelets and lymphocytes was evaluated in platelet activation studies and mixed lymphocyte cultures, respectively.

UVB source and dose calculations. The Haemonetics UV irradiator was equipped with a bank of nine UVB fluorescent tubes (Philips FST8) emitting a continuous spectrum between 270 and 340 nm with a maximum around 310 nm. Before irradiation, platelet and leukocyte suspensions (390 mL) were transferred to a Stericell® culture bag (Stericeal®; DuPont, Wilmington, DE; UVB transmission, 80%; cross-sectional layer depth, 4.3 mm) using the Sterile Docking Device from Haemonetics. The bags were placed on a quartz plate and irradiated from below under continuous agitation.
radiation (J/cm²) delivered on the cell suspensions was calculated as the product of the irradiance (mW/cm²) and the exposure times. UVB doses were corrected for the transmission through plastic material.

**Mixed lymphocyte cultures and platelet-activation studies.** Peripheral blood mononuclear cells (PBMCs) were prepared from freshuffy coats by density gradient centrifugation (Ficoll Isopaque; Pharmacia, Uppsala, Sweden). The cells were suspended in plasma (300 mL) at a concentration of 1 × 10⁶ cells/mL and transferred to Stericell bags for UVB irradiation. For dose-response studies, samples (3 mL) were taken from the bag during irradiation at different exposure times. In some experiments, leukocytes were irradiated in phosphate-buffered saline (PBS) at a concentration of 1 × 10⁶ cells/mL.

The effect of UVB irradiation on the ability of lymphocytes to stimulate responder cells was evaluated in the one-way MLR as reported before.13 In this assay, stimulator cells were also γ irradiated to block their allogeneic proliferative response. The ability of lymphocytes to stimulate in the MLR is not affected by γ irradiation. Lymphoproliferation induced by UVB-exposed stimulator cells was measured by incorporation of ³H-thymidine (specific activity, 2.1 GBq/mmol; Radiochemical Centre, Amersham, UK) and expressed as a percentage of control.

For platelet activation studies, leukodepleted platelets were collected fromuffy coats as reported before.16 In this study, platelets were collected from 18 buffy coats and pooled to obtain a PC with a volume of 900 mL. This PC was equally divided over three Stericell bags; one PC served as control, and two PCs were exposed to either 1.5 or 3.0 J/cm² in the Haemonetics UV irradiator. After UV exposure PCs were stored in 1000-mL Fenwal PL-732 bags (Baxter Healthcare, Fenwal Division, Deerfield, IL) for 96 hours at 22°C on a Helmer shaker (Helmer Labs, Noblesville, IN). UV-induced platelet activation was evaluated by measuring platelet counts, lactate levels, pH, and the expression of platelet-membrane activation markers during postirradiation storage.18 Flow cytometry was done in a FACScan flow cytometer (Becton Dickinson, Mountain View, CA) with platelets fixed with paraformaldehyde and stained with fluorescein isothiocyanate (FITC)-labeled MoAbs (RUU-PL 6.66 reacting with glycoprotein Ib and MoAb RUU-SP 2.15 specific for P-selectin).

**Clinical Study**

**Patients and transfusion policy.** Between October 1990 and September 1992, a total of 557 consecutive male patients undergoing elective surgery for coronary bypass grafting or valve replacement requiring cardiopulmonary bypass were selected for the study. Only patients with a history of blood transfusions were excluded to avoid secondary HLA-AI. The patients were transfused on clinical indication withuffy coat-depleted RBC concentrates, leukocyte depleted by filtration, and with pooled platelets prepared from 12 buffy coats. All blood components were delivered by the Red Cross Bloodbank Utrecht. Leukocyte depletion of RBCs was performed by filtration with BP4 filters (Fall Biomedical, Glencoe, NY) in a closed system. Leukocyte counts, measured in quality-control procedures, were always below 1 × 10⁶ cells/L.17 Pooled PCs were prepared within 12 hours after collection. The average leukocyte count in PCs prepared from 6 buffy coats was 2 × 10⁶ cells.17 Fresh PCs were transferred to Stericell bags for UVB irradiation, stored in Fenwall PL 732 bags, and transfused within 24 hours after preparation.

**Anti-HLA antibody screening.** Patients transfused with RBCs and/or PCs were selected for follow-up. From these patients, serum samples were taken before operation 1 week and 3 to 6 weeks after operation and kept at −70°C until tested for the presence of anti-HLA antibodies in the complement-dependent lymphocytotoxicity assay against a panel of 30 selected donors covering most of the defined HLA-A and B specificities.18 For the Ig class determination, diithiothreitol (DTT) was used. A serum with a positive reaction in the panel that turns into a negative reaction after IgM reduction with DTT is considered to contain IgM antibodies only. A positive panel reactivity despite DTT treatment indicates the presence of IgG antibodies. Anti-HLA antibodies were considered positive when the percentage of lysed cells per well was above 25% and when panel reactivity was at least 10%.

**Effects of UVB Radiation on Leukocytes**

**Detection of thymine dimers.** UVB radiation of cells induces alterations in DNA; the formation of cyclobutyl thymine dimers (T<>T) is very paramount. The amount of T<>T in DNA can be measured by flow cytometry using a MoAb with a mean fluorescence (FITC), against T<>T, as described by Berg et al.19 For accurate measurements we only detected T<>T in nuclei with 2 n DNA. For this purpose, DNA was stained with the red fluorescent dye 7-amino-actinomycin D (7-AAD) that allowed gating for nuclei with 2 n DNA.

Analysis of T<>T was done in PBMCs. The cells were suspended in plasma or in some experiments in PBS, irradiated in the Haemonetics UV irradiator and subsequently fixed in ice-cold 96% ethanol (1 × 10⁶ cells/mL). For analysis of T<>T in DNA, fixed cells were sedimented and incubated with 0.02% pepsin in 2 N HCl for 40 minutes to make the DNA accessible to the H3-Moab. Incubation was terminated with an excess of 0.1 mol/L Na₂B₄O₇. Nuclei were then incubated with 100 µL PBS containing 5% fetal calf serum (FCS) and 0.5% Tween 20 with 2.5% culture supernatant of the H3-MoAb. Bound H3-MoAbs were stained with 100 µL 1% vol/vol FITC-conjugated rabbit-antimouse IgGs (Dakopatts, Copenhagen, Denmark). Isootype- and fluorochrome-matched control MoAbs were used in each experiment to determine nonspecific background Moab binding. The nuclei were also incubated for 30 minutes at 37°C with 25 µg/ml 7-AAD in PBS to stain DNA. Fluorescence (10,000 events) was analyzed in the flow cytometer; FITC fluorescence was detected in the FL1 channel (530 ± 15 nm), 7-AAD fluorescence was recorded in the FL3 channel (>650 nm). Gates were set in the 7-AAD fluorescence to select events containing 2 n DNA (>80% of all events).

**Measurement of PBMC viability.** Viability was determined in PBMCs, T-cell-depleted with AET-treated sheep RBCs. The cells were suspended in 300 mL PBS or plasma at a concentration of 1 × 10⁶/mL, and UV-irradiated with the Haemonetics UV irradiator. Control and UVB samples (20 mL) were washed twice and suspended in RPMI 1640 (GIBCO, Life Technologies, Ltd, Paisley, UK) supplemented with 2% FCS and 1% human serum albumin (cell concentration 2 × 10⁶/mL). Each sample (12 mL) was then divided over three handmade Teflon bags (4 mL/bag; Fluorplast, Raamsdonksveer, The Netherlands) to avoid adherence of monocytes and cultured (5% CO₂; 37°C; humidified air) for up to 3 days. For each dose of UVB, one of the bags was opened daily and the number of viable cells was counted by trypsin blue dye exclusion. Viability and cell count of control suspensions did not change during incubation. The number of viable cells in UV-exposed suspensions was expressed as a percentage of age-matched control.

**Statistical analyses.** Differences in the expression of surface markers between UV-irradiated platelets and age-matched control platelets were analyzed with the paired t-test. Differences in the
by the Haemonetics UV irradiator. Table 2 is based on the findings of a study conducted by the Haemonetics UV irradiator.

Addition of platelets (3 x 10^11/mL) to the leukocyte suspensions did not change the dose of UVB required for abolition of alloreactive responses and indicates that platelets do not interfere with the effect of UVB on leukocytes suspended in plasma.

Recent studies have shown that further increase of the dose results in progressive activation of platelets during postirradiation storage at 3, 48, and 96 hours. The samples were analyzed for platelet count, pH, lactate, and surface expression of P-selectin. Table 2 shows that 3.0 J/cm^2 of UVB induces a progressive increase in activated platelets during storage. The dose of 1.5 J/cm^2 did not affect biochemical parameters and expression of P-selectin on the platelets during storage for up to 96 hours, and was selected for clinical use.

Clinical Study

The effect of UVB irradiation on the incidence of HLA alloimmunization was evaluated in a prospective controlled clinical study, performed in cardiac patients undergoing cardiopulmonary bypass. A total of 557 consecutive male patients was selected for the study; 372 patients were not transfused, 22 patients were transfused only with plasma, 62 patients were transfused with plasma and filtered RBCs, and 101 patients were transfused with plasma, filtered RBCs, and platelets. Patients were only transfused on clinical indication. If platelets were required, the patients were transfused with a single transfusion of pooled platelets prepared from a total of 12 donors. Platelets were either standard or UVB irradiated, but never filtered or γ irradiated. Anti-HLA antibody screens were performed before operation, 1 week after operation and 3 to 6 weeks after operation in patients transfused with cellular blood components. Patients with anti-HLA antibodies before operation were excluded for further evaluation. In the group of patients transfused with plasma and RBCs, 53 patients received an average of 2.1 U (range, 1 to 4 U) of RBCs and 9 patients received an average of 8.5 U (range, 6 to 11 U) of RBCs. In all these patients, anti-HLA antibody screens were negative. In the group of patients transfused with platelets, 39 were transfused with standard PCs (control group) and 62 patients were transfused with UV irradiated PCs (UVB group). In the control group, 17 patients were transfused with an average of 4.3 ± 2.5 U (SD) of RBC (range, 1 to 8 U) and 5 patients received two transfusions of PCs prepared from 12 donors each. In the UVB group, 29 patients were transfused with an average of 3.7 ± 2.5 U (SD) of RBCs (range, 1 to 11 U) and 2 patients received two transfusions of PCs prepared from 12 donors each. Table 3 shows transfusion characteristics of the patients who developed anti-HLA antibodies. Figure 1 shows the panel reactivity of the antibodies in each patient; the presence of IgG anti-HLA antibodies alone was evaluated in sera after IgM reduction with DDT. Patient 2 in the control group developed auto-antibodies; panel reactivity was 100% after IgM reduction with DDT. Thus, the incidence of alloantibodies against HLA antigens is 12.8% (of 39 patients) in the control group and 9.7% (of 62 patients) in the UVB group (P = .62). Induction of IgM anti-HLA antibodies was found in 4 patients in the control group and 5 patients in the UVB group. Specificity of anti-HLA antibodies is listed in Table 3.

Effects of UVB Radiation on Leukocytes

Association between loss of stimulatory activity in the MLR and amount of thymine dimers in DNA. The outcome

Table 1. The Effect of UV Radiation on Ability of Lymphocytes to Stimulate in MLR

<table>
<thead>
<tr>
<th>Dose (J/cm^2)</th>
<th>3HdR-Incorporation (% of control)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.12</td>
<td>39 ± 13</td>
</tr>
<tr>
<td>0.25</td>
<td>15 ± 5</td>
</tr>
<tr>
<td>0.37</td>
<td>5 ± 2</td>
</tr>
<tr>
<td>0.50</td>
<td>&lt;1</td>
</tr>
</tbody>
</table>

PBMCs were suspended in plasma and exposed to increasing doses of UVB. Data are means ± SEM (n = 6).

RESULTS

Determination of the Optimal Dose of UVB Radiation

For successful irradiation of PCs, it is essential to select a dose that abolishes the ability of passenger leukocytes to stimulate in the MLR and spares the hemostatic function of the platelets. In this study, PBMCs, suspended in 300 mL plasma, were exposed to increasing doses of UVB delivered by the Haemonetics UV irradiator. Table 1 shows that a dose of 0.5 J/cm^2 was sufficient to abolish alloreactive responses in the MLR (responder cell proliferation below 1% of control). Addition of platelets (3 x 10^11/mL) to the leukocyte suspensions did not change the dose of UVB required for abolition of alloreactive responses and indicates that platelets do not interfere with the effect of UVB on leukocytes suspended in plasma.

Clinical Study

The effect of UVB irradiation on the incidence of HLA alloimmunization was evaluated in a prospective controlled clinical study, performed in cardiac patients undergoing cardiopulmonary bypass. A total of 557 consecutive male patients was selected for the study; 372 patients were not transfused, 22 patients were transfused only with plasma, 62 patients were transfused with plasma and filtered RBCs, and 101 patients were transfused with plasma, filtered RBCs, and platelets. Patients were only transfused on clinical indication. If platelets were required, the patients were transfused with a single transfusion of pooled platelets prepared from a total of 12 donors. Platelets were either standard or UVB irradiated, but never filtered or γ irradiated. Anti-HLA antibody screens were performed before operation, 1 week after operation and 3 to 6 weeks after operation in patients transfused with cellular blood components. Patients with anti-HLA antibodies before operation were excluded for further evaluation. In the group of patients transfused with plasma and RBCs, 53 patients received an average of 2.1 U (range, 1 to 4 U) of RBCs and 9 patients received an average of 8.5 U (range, 6 to 11 U) of RBCs. In all these patients, anti-HLA antibody screens were negative. In the group of patients transfused with platelets, 39 were transfused with standard PCs (control group) and 62 patients were transfused with UV irradiated PCs (UVB group). In the control group, 17 patients were transfused with an average of 4.3 ± 2.5 U (SD) of RBC (range, 1 to 8 U) and 5 patients received two transfusions of PCs prepared from 12 donors each. In the UVB group, 29 patients were transfused with an average of 3.7 ± 2.5 U (SD) of RBCs (range, 1 to 11 U) and 2 patients received two transfusions of PCs prepared from 12 donors each. Table 3 shows transfusion characteristics of the patients who developed anti-HLA antibodies. Figure 1 shows the panel reactivity of the antibodies in each patient; the presence of IgG anti-HLA antibodies alone was evaluated in sera after IgM reduction with DDT. Patient 2 in the control group developed auto-antibodies; panel reactivity was 100% after IgM reduction with DDT. Thus, the incidence of alloantibodies against HLA antigens is 12.8% (of 39 patients) in the control group and 9.7% (of 62 patients) in the UVB group (P = .62). Induction of IgM anti-HLA antibodies was found in 4 patients in the control group and 5 patients in the UVB group. Specificity of anti-HLA antibodies is listed in Table 3.

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Table 2. Effect of UVB-Radiation on Platelet Activation With Storage

<table>
<thead>
<tr>
<th>Storage Time (hrs)</th>
<th>Control</th>
<th>1.5 J/cm^2</th>
<th>3.0 J/cm^2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Platelet count (% of initial)</td>
<td>97 ± 4</td>
<td>96 ± 4</td>
<td>96 ± 4</td>
</tr>
<tr>
<td>pH</td>
<td>7.30 ± 0.03</td>
<td>7.30 ± 0.02</td>
<td>7.29 ± 0.04</td>
</tr>
<tr>
<td>Lactate (mmol/L)</td>
<td>5.1 ± 0.4</td>
<td>5.1 ± 0.2</td>
<td>5.5 ± 0.5</td>
</tr>
<tr>
<td>P-selectin (%)</td>
<td>22 ± 1</td>
<td>21 ± 2</td>
<td>22 ± 2</td>
</tr>
</tbody>
</table>

Platelets exposed to UVB doses of 1.5 and 3.0 J/cm^2 and control platelets were stored for up to 96 hours. Data are means ± SEM, n = 6.

* P < .01 (paired t-test for age-matched data).
Table 3. No. of Transfusions and Specificities of Panel-Reactive Antibodies

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>RBCs* (donors)</th>
<th>PCst (12 pool)</th>
<th>Plasma (donors)</th>
<th>Anti-HLA Antibodies (specificities)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control group</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>5</td>
<td>1</td>
<td>8</td>
<td>B18</td>
</tr>
<tr>
<td>2</td>
<td>-</td>
<td>1</td>
<td>2</td>
<td>-</td>
</tr>
<tr>
<td>3</td>
<td>1</td>
<td>1</td>
<td>-</td>
<td>A2, A28</td>
</tr>
<tr>
<td>4</td>
<td>-</td>
<td>1</td>
<td>-</td>
<td>B12</td>
</tr>
<tr>
<td>5</td>
<td>-</td>
<td>1</td>
<td>-</td>
<td>Broad reactive</td>
</tr>
<tr>
<td>6</td>
<td>5</td>
<td>2</td>
<td>6</td>
<td>A11, B17</td>
</tr>
<tr>
<td>UVB group</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>6</td>
<td>2</td>
<td>4</td>
<td>B17</td>
</tr>
<tr>
<td>2</td>
<td>-</td>
<td>1</td>
<td>2</td>
<td>A1, A9</td>
</tr>
<tr>
<td>3</td>
<td>4</td>
<td>1</td>
<td>2</td>
<td>B7, B13</td>
</tr>
<tr>
<td>4</td>
<td>1</td>
<td>1</td>
<td>-</td>
<td>A2, A28</td>
</tr>
<tr>
<td>5</td>
<td>2</td>
<td>1</td>
<td>-</td>
<td>Broad reactive</td>
</tr>
<tr>
<td>6</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>Broad reactive</td>
</tr>
</tbody>
</table>

Depicted are all patients with positive anti-HLA antibody screens after operation.
* No. of red blood cell concentrates prepared from one donor.
† No. of platelet concentrates prepared from 12 donors.

The clinical study indicates that transfusion of UVB-irradiated PCs does not reduce the incidence of HLA-AI. For better understanding of this finding we measured the amount of UVB-induced T'<><>T in DNA of individual cells. This was done with a novel flow-cytometric technique that became available to us at the end of the clinical study. The histograms shown in Fig 2 are measurements of PBMCs prepared from one donor and are representative for a series of experiments. Figure 2A shows T'<><>T histograms of UVB-irradiated leukocytes that were suspended in plasma during the irradiation. The distribution of dimers in these cells is wide and indicates the large variation in UVB-induced damage per cell, which can be ascribed to a combination of poor mixing of cells and poor transmission of UVB radiation through plasma. In subsequent experiments (Fig 2B), we suspended leukocytes in PBS (good UVB transmission) and exposed these suspensions to UVB to establish more precisely the level of T'<><>T that corresponds to the loss of stimulatory responses in the MLR. As expected, histograms are narrow indicating that the variation in the amount of dimers in the cells is small. Mean fluorescence intensity (MFI) increased linearly with the dose of UVB. The capacity of these cells to stimulate the MLR is shown in Table 4. The amount of dimers in the cells exposed to 36 ml/cm² corresponds to complete loss of alloreactivity in the MLR. Figure 2C is a composite plot of two T'<><>T histograms obtained from leukocytes suspended in plasma and exposed to 0.5 and 1.5 J/cm² and one T'<><>T histogram obtained from leukocytes suspended in PBS and exposed to 36 ml/cm². Leukocytes exposed to 0.5 J/cm² (in plasma) and 36 ml/cm² (in PBS) had completely lost their ability to induce alloreactive responses in the MLR. About 30% of the cells suspended in plasma and exposed to 0.5 J/cm² showed T'<><>T levels, which were below the level of dimers found in the cells exposed to 36 ml/cm². All leukocytes exposed to the clinical dose (1.5 J/cm²) showed T'<><>T levels which were equal or above the level of dimers found in cells exposed to 36 ml/cm² and indicates that this clinical dose should have been adequate to render all antigen-presenting cells dysfunctional.

Association between UVB dose, amount of thymine dimers, and viability. Several studies have shown that UVB radiation induces loss of viability in a dose-dependent manner. Measurement of T'<><>T enables us to associate the level of DNA damage with cell death. T-cell–depleted PBMCs were suspended in saline and exposed to 6, 12, 36, and 72 ml/cm² of UVB. After UV exposure, the cells were cultured in Teflon bags for up to 3 days. Every 24 hours, aliquots were taken from control and UV-exposed suspensions to determine viability. Table 4 shows the correlation between the amount of dimers (arbitrary units) in the cells after UVB exposure and the loss of viability for culture for 3 days. Each dose of UVB induced a progressive decrease in viability over time. The dose of 36 ml/cm² resulted in abolition of alloreactivity in the MLR and in less than 5%...
of viable cells after 72 hours of culture. We also determined viability of cells suspended in plasma and exposed to 0.5 and 3.0 J/cm². The dose of 0.5 J/cm² resulted in loss of alloreactivity in the MLR, but not in complete loss of viability; 25% of cells were still viable after 3 days of culture. It is plausible that part of the cells with T< >T levels below the levels found in cells exposed to 36 mJ/cm² recover from the deleterious effects of UVB and remain viable. The clinical dose of 3.0 J/cm² resulted in less than 5% of viable cells after 24 hours of culture. The MFI in these cells was 310 ± 41 arbitrary units because of the wide distribution of dimers in these cells. Almost all cells showed T< >T levels that were equal or above the level of dimers found in cells suspended in saline and exposed to 36 mJ/cm² of UVB (Fig 2).

**DISCUSSION**

In this report, we have evaluated the administration of UVB-irradiated PCs for the prevention of HLA allosensitization. All previous studies from our laboratory were designed to determine the optimal dose of UVB were performed with an experimental irradiation cabinet. In this study, PCs were exposed to UVB radiation delivered by the UV irradiator provided by Haemonetics Corp. We found that a dose of 0.5 J/cm² was needed to abolish alloreactivity of passenger leukocytes in MLR (Table 1). This observation is in concert with data from other studies. Pamphilon et al and Andreu et al exposed PCs to similar sources of UVB and reported that 0.3 to 0.5 J/cm² of UVB was sufficient to abolish MLR alloreactivity.

The selection of the dose of UVB for irradiation of PCs in the clinical study was made on the basis of data obtained from studies on APCs. The most striking effect of UVB is the inhibition of APCs to stimulate responder cells in the allogeneic MLR. In this assay, class II major histocompatibility complex (MHC) determinants on the APCs bind to the T-cell receptor in an antigen-specific manner. As a consequence, the ability of UVB-exposed cells to stimulate responder cells was evaluated in the MLR. All previous studies from our laboratory that were designed to determine the optimal dose of UVB were performed with an experimental irradiation cabinet. In this study, PCs were exposed to UVB radiation delivered by the UV irradiator provided by Haemonetics Corp. We found that a dose of 0.5 J/cm² was needed to abolish alloreactivity of passenger leukocytes in MLR (Table 1). This observation is in concert with data from other studies. Pamphilon et al and Andreu et al exposed PCs to similar sources of UVB and reported that 0.3 to 0.5 J/cm² of UVB was sufficient to abolish MLR alloreactivity.

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### Table 4. Effect of UVB Radiation on Alloreactivity, DNA Damage, and Viability

<table>
<thead>
<tr>
<th>UVB Dose (mJ/cm²)</th>
<th>³HThdR Incorporation (control %)</th>
<th>TOT (arbitrary units)</th>
<th>Viability (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>24 h</td>
</tr>
<tr>
<td>6</td>
<td>48 ± 13</td>
<td>82 ± 7</td>
<td>63 ± 3</td>
</tr>
<tr>
<td>12</td>
<td>26 ± 3</td>
<td>38 ± 5</td>
<td>27 ± 2</td>
</tr>
<tr>
<td>18</td>
<td>15 ± 5</td>
<td>29 ± 6</td>
<td>16 ± 4</td>
</tr>
<tr>
<td>24</td>
<td>9 ± 4</td>
<td>34.3 ± 1.5</td>
<td></td>
</tr>
<tr>
<td>30</td>
<td>4 ± 3</td>
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<td></td>
</tr>
<tr>
<td>36</td>
<td>&lt;1</td>
<td>47.0 ± 2.3</td>
<td>16 ± 5</td>
</tr>
<tr>
<td>72</td>
<td>11 ± 3</td>
<td>113 ± 1</td>
<td>3 ± 1</td>
</tr>
</tbody>
</table>

PBMCs suspended in PBS were exposed to increasing doses of UVB. The ability of UVB-exposed cells to stimulate responder cells was evaluated in the MLR. Alloreactivity was expressed as a percentage of control. DNA damage and viability were evaluated in T-cell-depleted PBMCs. For evaluation of DNA damage, the level of thymine dimers (TOT) was measured by flow cytometry and expressed in arbitrary units. Viability of UVB-exposed cells was determined after culture for up to 72 hours and expressed as a percentage of age-matched controls. Data are means ± SEM (n = 6).
quence, costimulatory signals are transmitted from the APCs to the T-cells. UV irradiation interferes with a number of processes involved in the MLR. Studies in plastic adherent blood mononuclear cells, which are enriched for monocytes, showed that low doses of UVB (5 mJ/cm²) are deleterious to their accessory function in antigen and mitogen-induced T-cell responses.20 In later studies from the same laboratory, Krutmann et al24 evaluated monocyte accessory cell function in supporting anti-CD3 mitogenesis. They described a dose-dependent reduction in intercellular adhesion molecule 1 (ICAM-1) surface expression on UVB-irradiated monocytes associated with a corresponding decrease in cluster formation and T-cell proliferation. A dose-dependent recovery of ICAM-1 expression was seen 1 to 3 days after UV irradiation, indicating that viability of the cells was not impaired.25 However, higher doses of UVB reduced the viability of adherent mononuclear cells in proportion to the dose administered. Based on these considerations, we decided to select the highest possible dose in order to induce irreversible inactivation of the leukocytes. According to Table 2, a dose of 1.5 J/cm² did not affect the platelets and was found most appropriate.

Recently, Young et al26 evaluated the sensitivity of human blood dendritic cells (DCs) for UVB radiation. At given doses of UVB, DCs were found to be more resistant than monocytes or B-cells in reducing alloreactive T-cell responses in the MLR. UVB irradiation of DCs prevented the upregulation of costimulatory ligands B7/BB1 and ICAM-1/CD54 in a dose-dependent manner after alloreactive T-cell binding, but did not affect cluster formation between DCs and T-cells during the first 1 to 2 days of the allogeneic MLR. UVB-exposed cells remained viable and class II MHC molecule expression was not decreased. Although UVB prevented upregulation of ICAM-1 and B7/BB1 expression on DCs in the clusters, interleukin-2 (IL-2) receptor surface expression on individual T-cells was not affected. However, there was a profound diminution in T-cell autocrine IL-2 secretion, after their aggregation with UVB-irradiated DCs, in a dose-dependent manner. It is presumed that the abolition of T-cell proliferation in the MLR by UVB is caused by the low expression of B7/BB1 on DCs. The interaction between B7/BB1 and CD28 on T-cells is the only one known to stimulate T-cell proliferation by a direct effect on IL-2 gene transcription and production.27,28 Thus, for selection of the optimal dose, we should consider that DCs, which are the most potent stimulators, are more resistant to the deleterious effects of UVB than monocytes. We should also consider that UV-irradiated DCs with intact viability may recover from the deleterious effects of UVB and regain their ability to upregulate the expression of costimulatory ligands. These considerations warrant the use of the highest dose of UVB that does not induce platelet activation.

The clinical study showed that a single transfusion of pooled platelets (4 x 10⁹ leukocytes) induced HLA sensitization in 5 of 39 patients (12.8%). This incidence is rather low when compared with the incidence of alloimmunization in patients transfused with 1 U of leukocyte-poor blood (5 x 10⁹ leukocytes). In these studies, Lagaay et al29 found anti-HLA antibodies in 18 of 30 patients (60%) 2 weeks after transfusion. Data from this study suggests that the transfusion of UVB-irradiated platelets results in a trend towards lower sensitization rates (9.7%), taking into account that the numbers studied are small statistically. Preliminary results in patients with acute leukaemia, who were transfused with UV-exposed PCs and filtered RBCs, also suggested that UV irradiation may lead to lower sensitization rates.30,31

In view of the generally accepted concept, that viable donor APCs are responsible for the onset of HLA alloimmunization via the direct pathway of allorecognition, we did not expect that UVB-inactivated APCs were able to induce anti-HLA antibodies. For better understanding of this observation, we analyzed the effect of UVB on the cells by measuring the level of T<->T in DNA with a specific MoAb; a technique that came available to us at the end of the clinical study. The level of T<->T in the cells can easily be measured by flow cytometry and provides a tool for determining the association between the level of DNA damage and loss of viability. In control experiments, we first exposed cells suspended in PBS to different doses of UVB just to show that increasing levels of T<->T were associated with decreases in viability (Table 4). This measurement was used to show that the level of T<->T in cells suspended in plasma and exposed to the clinical dose of UVB (1.5 J/cm²) was above the level required for complete loss of viability in all cells. Despite these findings, we found production of anti-HLA antibodies in patients transfused with UVB-exposed platelets. For an explanation of this finding, we should consider triggering of the indirect pathway of allorecognition. Heavily UV-irradiated cells may disintegrate in the circulation and release large amounts of soluble class I HLA antigens, which are then processed and presented by host APCs. The antigen-specific pathway of allorecognition was also reported by Pellegrino et al.32 The authors found that transfusion of plasma containing leukocyte fragments from selected donors resulted in the onset of anti-HLA antibodies. Presentation of donor MHC peptides by host APCs resulting in CD4+ T-helper cell responses has also been found in murine immunization models.33,34 The amount of soluble class I HLA antigens released from the leukocytes is probably important. The clinical observation that platelets do not induce anti-HLA antibodies is most likely explained by the poor expression of class I HLA antigens on the platelet membrane. When platelets disintegrate, the release of these antigens probably remains below the threshold required for HLA alloimmunization.

We conclude from the data presented in this study that UVB irradiation of PCs results in a trend toward lower sensitization rates. The relatively high dose of UVB (1.5 J/cm²), used for inactivation of leukocytes in PCs, may induce fragmentation of cells and alloimmunization according to the indirect pathway of allorecognition. We propose that fragmentation of the cells is associated with the observation that the cells are exposed to a wide range of UVB energy, in concert with the wide distribution of UVB-induced DNA damage. As a result, large numbers of cells are heavily overexposed to UVB. To reduce the number of overexposed
cells, we would suggest UVB irradiation of cells suspended in a crystalloid medium. Recently, electrolyte solutions have been used for storage of platelet concentrates. These solutions allow the application of relatively low doses of UVB for inactivation of all leukocytes and reduction in the number of heavily overexposed cells.

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