Induction of Humoral Immune Tolerance to Major Histocompatibility Complex Antigens by Transfusions of UVB-Irradiated Leukocytes

By K.J. Kao

To determine whether immune tolerance to donor major histocompatibility complex (MHC) antigens can be induced by transfusions of UVB-irradiated leukocyte concentrates, studies were conducted in inbred strains of mice with well-characterized MHC antigens. CBA mice with H-2d phenotype and BALB/c mice with H-2d' phenotype were used as recipients and donors, respectively. Humoral immune tolerance is defined as absence of antibody response after challenge with transfusions of untreated donor leukocytes. It was found that transfusions of purified peripheral blood mononuclear leukocytes irradiated with 1,200 mJ/cm² UVB not only prevented alloimmunization but also induced humoral immune tolerance to donor class-I and -II MHC antigens in all recipient mice. Donor plasma and platelets interfered with the induction of this tolerance. The tolerance induction by UVB-irradiated leukocytes is dose-dependent. Four weekly transfusions of 2 × 10⁸ leukocytes were required to ensure tolerance induction in all mice. The results of a long-term follow-up study showed that the induced tolerance is long-lasting and can withstand repeated challenges by untreated donor leukocytes. Recipient mice tolerant to H-2d' antigens also became tolerant to H-2d', H-2', and H-2d MHC antigens but did not have impaired antibody responses to antigens unrelated to donor leukocytes. Adoptive transfer experiments showed the development of negative regulatory cells in spleens of the tolerant mice. In view of recent feasibility of using UVB-irradiated human platelet concentrates for prevention of HLA alloimmunization, the findings of this study support that UVB-irradiated donor leukocytes could have potential, important clinical application in transplantation medicine.

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Prevention of transfusion- or transplantation-induced primary alloimmunization to donor major histocompatibility complex (MHC) antigens can be accomplished by eliminating leukocytes in either donor blood or allografts. In addition, it has been recognized that MHC antigens on isolated cell membranes or in plasma are poorly immunogenic. Therefore, intact and functioning donor leukocytes, rather than donor MHC antigens per se, are believed to be responsible for alloimmunizing transfusion recipients to donor MHC antigens.

Another approach for preventing transfusion-induced HLA alloimmunization is the use of UV light to inactivate donor leukocytes in platelet concentrates. As shown in animal studies, irradiation of platelet concentrates with medium-wavelength UV light (290 to 320 nm; UVB) is more efficacious than leukocyte depletion for prevention of alloimmunization to donor MHC antigens. It has been documented that both UVB and UVC (260 to 290 nm wavelength) have a wide range of immunomodulatory effects, including inhibition of stimulator and responder function of leukocytes in mixed leukocyte cultures (MLCs), downregulation of cell membrane proteins such as class-II MHC antigens and cell adhesion molecules, suppression of cytokine production, and inhibition of lymphocyte proliferative responses to mitogenic lectins. Irradiation of pancreatic islets, skin allografts, and allogeneic donor bone marrow by UVB or UVC also has been shown to prolong the survival of allografts or to prevent graft-versus-host disease in various animal studies.

In addition to the reduction of alloantigenicity and prevention of graft-versus-host disease, transfusions of UV-irradiated blood components are able to induce immune tolerance to donor MHC antigens. For instance, transfusions of UVB-irradiated platelet concentrates not only prevented transfusion refractoriness to donor platelets in 92% of recipient dogs, but also induced immune tolerance to donor platelets in 70% of the recipients. However, in mice, partial immune tolerance to donor MHC antigens was induced with UVB-irradiated platelet concentrates. However, the induction of tolerance by transfusions of UV-irradiated platelet concentrates has been either incomplete or, sometimes, unsuccessful. Factors important for consistent induction of immune tolerance by UV-irradiated donor blood components apparently exist and remain to be identified. The cellular component(s) that is responsible for tolerance induction in UV-irradiated blood products also remains to be shown.

Prompted by the recent feasibility of preparing UVB-irradiated blood components for patient use and the clinical importance of tolerance induction for transplantation of allografts, a series of studies were conducted to identify cellular component and factors important for the reliable induction of humoral immune tolerance to donor MHC antigens. Additional studies were performed to characterize the tolerance induced by UVB-irradiated leukocytes.

Materials and Methods

Animals, hybridomas, and cell lines. Eight-week-old inbred CBA/CaH-T6J(CBA) mice with H-2d MHC haplotype and BALB/cByJ (BALB/c) mice with H-2d' MHC haplotype were purchased from Jackson Laboratory (Bar Harbor, ME). BALB/c mice were used as blood donors, and CBA mice were used as transfusion recipients. All mice were housed in a temperature-controlled room (25°C) with a 12-hour interval light/dark cycle and were fed ad libitum. All animal studies were approved by the Institutional Animal Care and Use Committee. The 34-1-2s hybridoma known to produce a murine IgG2aκ monoclonal antibody (MoAb) to class I H-2d MHC antigens was obtained from American Type Culture Collection (Rockville, MD). The hybridoma cells were maintained in the presence of 10% fetal bovine serum in RPMI 1640 medium, and the hybridoma supernatants were collected twice a week for analysis of antibody levels.

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in Dulbecco’s modified Eagle’s medium containing 10% fetal calf serum, 60 µg/mL gentamicin, and 1% antibiotic–antimycotic solution (GIBCO Laboratories, Grand Island, NY). HMY/CIR cell line derived from human plasma cell leukemia was transfected with cDNAs for α and β chains of murine IEd class-I MHC molecules. Both cDNAs used for transfection were cloned into a pcDNA3 expression vector (Invitrogen, San Diego, CA). This transfected cell line that expresses IEd class-I MHC molecules was a generous gift of Dr E.K. Wakeland (Department of Pathology, University of Florida, Gainesville).

Preparation of platelet concentrates, plasmas, and mononuclear leukocytes (MNLs). Platelet concentrates and peripheral blood MNLs were prepared from freshly collected venous blood by differential and Ficoll-hypaque gradient centrifugation as described previously.18 Concretions of platelets and MNLs were determined by a Coulter ZM counter (Coulter Corp, Hialeah, FL) and by a propidium iodide staining method, respectively.10

UVB and γ-irradiation. A Bioslink-UV irradiator (BIOS Corp, New Haven, CT) with a built-in dosimeter was used for irradiation. The wavelength of peak UV light emission was 312 nm. The dose of UVB used to irradiate leukocytes and platelets was 1,200 mJ/cm². The viability of leukocytes 1 hour after UVB irradiation was always greater than 98% as determined by trypan blue staining. UVB irradiation was performed in an open sterile polypropylene container. The depth of cell suspension was ±2 mm. Cells were suspended in phosphate-buffered saline (PBS) and mixed manually by moving the tray back and forth once per second during irradiation. γ-Irradiation was performed in an IBL-437C irradiator (CTS-US, Inc, Bedford, MA). Stimulator cells were irradiated with 25 Gy γ-rays before the MLC experiments.

Platelet transfusion experiments. Each recipient mouse was transfused with donor leukocytes and/or platelets in a volume of 100 µL once per week through a tail vein under light anesthesia with inhalation of methoxyflurane (Pitman-Moore, Inc, Mundelein, IL). The wavelength of peak UVB used to irradiate leukocytes and platelets was 1,200 mJ/cm². The dose of γ-irradiation was always greater than 98% as determined by trypan blue staining. The irradiation procedure for detecting antibodies to class-I H-2d antigens was described above.

Enzyme-linked immunosassay (EIA) for detecting antibodies to class-I H-2d antigens. To show the presence of antibodies to class-I H-2d molecules in the sera of recipient mice, serum samples were assayed by an EIA. For this assay, plastic wells of a microtiter plate were coated with 50 µL of 0.75 µg/mL class-I H-2d molecules diluted in PBS containing 0.02% sodium azide. Class-I H-2d molecules were isolated from spleen cells of BALB/c mice. The cells were solubilized with 100 mL of 0.02 mol/L Tris buffer (pH 7.4), containing 2% NP-40, 5 mmol/L EDTA, 1 mmol/L phenylmethylsulfonyl fluoride, 4 mmol/L epsilon-aminocaproic acid, and 1 mmol/L diisopropyl fluorophosphate. After centrifugation of the lysate at 20,000g at 4°C for 60 minutes to remove insoluble materials, class-I H-2d molecules were isolated from the supernatant using the 34-1-2s anti-H-2d MoAb covalently coupled to CNBr-activated Sepharose 4B (Sigma Corp). The procedure of affinity-column chromatography was the same as that for isolating HLA antigens.25 The protein concentration of the purified class-I H-2d molecules was determined by a modified Lowry’s assay26 using BSA as a protein concentration standard. After coating with class-I H-2d molecules, each well was blocked with 1% BSA, and incubated sequentially with 5X diluted mouse serum, alkaline phosphatase conjugate of goat antimouse IgG antibody, and alkaline phosphatase substrate. The results of the EIA procedure for detecting antibodies to class-I H-2d molecules was similar to what had been reported for anti-HLA antibodies.27

Humoral immune responses to T-cell-dependent and -independent antigens. To study the humoral immune responses to antigens unrelated to MHC molecules, each CBA mouse was immunized with chicken ovalbumin (Sigma Corp), a T-cell-dependent antigen, or a polyclonal pneumococcal vaccine (Pneu-Immune-23; Lederle Laboratories, Pearl River, NY), a T-cell–independent antigen. Mice were injected intraperitoneally with 0.1 mL ovalbumin or pneumococcal vaccine on day 1 and day 10. Ovalbumin was dissolved in PBS at a concentration of 1 mg/mL. Serum samples were collected on days 0, 10, and 18. To detect antibodies to pneumococcal polysaccharides, each well of an EIA assay plate was coated with 50 µL pneumococcal vaccine diluted to 25 µg/mL in PBS-azide overnight at 4°C. Antibodies to ovalbumin were detected in wells coated with 50 µL ovalbumin (10 µg/mL in PBS-azide). After washing and blocking with 1% BSA, each well was incubated with 50 µL of 10X diluted mouse serum at room temperature for 45 minutes. The bound antibodies were detected with alkaline phosphatase conjugate of goat antimouse IgG whole molecule antibodies and substrate (Sigma Corp).

Adoptive transfer studies. Spleen MNLs were isolated by Ficoll-hypaque gradient centrifugation from naive CBA mice and from CBA mice immunized or tolerant to BALB/c donor MHC antigens.28 The MNLs were washed with 10 mL sterile PBS 3 times and resuspended in 0.5 mL PBS. Each naive CBA mouse was infused through a tail vein with 30 X 10⁶ spleen mononuclear cells suspended in 150 µL PBS. Beginning 3 days after receiving these spleen cells, each CBA mouse was challenged with 4 weekly transfusions of 4 X 10⁶ untreated BALB/c mice peripheral blood MNLs. Serum samples from the CBA mice were collected 2 and 4 weeks after the first BALB/c MNL transfusion challenge.

RESULTS
Effect of plasma and platelets on the induction of humoral immune tolerance by UVB-irradiated leukocytes. Naïve male CBA mice were treated with 4 weekly transfusions of BALB/c peripheral blood MNLs that were suspended in (1)
Plasma and platelets can interfere with tolerance induction by UVB-irradiated donor leukocytes. In addition, it was found that resuspending donor leukocytes with minimal contamination of plasma and platelets is critical for induction of immune tolerance.

Characterization of antibodies in alloimmunized recipients. Serum samples of mice transfused with untreated donor MNLs were characterized for the presence of antibodies to both class-I and -II H-2d antigens using purified class-I H-2d molecules and a transfected human cell line expressing murine class-II IE3 MHC molecules. The results show that all mice transfused with untreated donor leukocytes developed antibodies to both class-I and -II donor MHC antigens (Table 2). Therefore, recipient mice transfused with UVB-irradiated leukocytes suspended in PBS indeed became tolerant to both class-I and -II donor MHC antigens.

Dose dependency of tolerance induction. To learn whether the induction of humoral immune tolerance by UVB-irradiated donor leukocytes is dose-dependent or not, 35 CBA mice were randomly divided into five groups and

Table 1. Effect of Platelets and Plasma on UVB-irradiation to Inactivate Stimulator and Responder Function of BALB/c Mice

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Stimulator Activity (cpm)*</th>
<th>Responder Activity (cpm)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unirradiated control</td>
<td>21,501 ± 321 (100%)</td>
<td>43,165 ± 2,911 (100%)</td>
</tr>
<tr>
<td>UVB-WBC</td>
<td>607 ± 62 (2.6%)</td>
<td>483 ± 80 (1.1%)</td>
</tr>
<tr>
<td>UVB-(PRP + WBC)</td>
<td>510 ± 24 (2.4%)</td>
<td>354 ± 140 (0.8%)</td>
</tr>
<tr>
<td>UVB-(PPP + WBC)</td>
<td>727 ± 186 (3.4%)</td>
<td>233 ± 98 (0.5%)</td>
</tr>
</tbody>
</table>

Abbreviations: UVB-WBC, leukocytes suspended in PBS and irradiated with UVB; UVB-(PRP + WBC), leukocytes suspended in PRP and irradiated with UVB; UVB-(PPP + WBC), leukocytes suspended in PPP and irradiated with UVB.

* Incorporation of tritiated thymidine by responder leukocytes in MLC. Each value is a mean of triplicate incubations (mean ± SD).

Table 2. Development of Antibodies to Class-I and -II H-2d MHC Molecules in CBA Mice Transfused with BALB/c Peripheral Blood MNLs

<table>
<thead>
<tr>
<th>Serum Samples (n = 4)</th>
<th>Antibodies to Class-I H-2d Antigens (OD\textsubscript{405nm})*</th>
<th>Antibodies to Class-II H-2d Antigens (fluorescence intensity)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Preimmune sera</td>
<td>0.077, 0.060</td>
<td>40, 49</td>
</tr>
<tr>
<td></td>
<td>0.059, 0.049</td>
<td>40, 45</td>
</tr>
<tr>
<td></td>
<td>(0.061 ± 0.098)†</td>
<td>(43 ± 4)</td>
</tr>
<tr>
<td>Immune sera‡</td>
<td>0.581, 0.603</td>
<td>425, 634</td>
</tr>
<tr>
<td></td>
<td>0.897, 0.735</td>
<td>337, 136</td>
</tr>
<tr>
<td></td>
<td>(0.669 ± 0.098)‡</td>
<td>(383 ± 205)</td>
</tr>
</tbody>
</table>

Abbreviation: OD\textsubscript{405nm}, optical density at 405 nm.

* Anti-class-I antibodies were measured by EIA using the purified class-I H-2d antigens. Anti-class-II antibodies were assayed by immunofluorescence flow cytometry using human HMY-CIR cell line expressing murine class-II IE3 antigens as target cells.
† Mean ± SD.
‡ Immune sera were collected from CBA mice after receiving 4 weekly transfusions of BALB/c peripheral blood MNLs (2 × 10^5 cells/transfusion).
recipient mice became immunized. In a separate experiment, controls to ensure that the transfused donor leukocytes were untreated BALB/c donor leukocytes (2 \times 10^5 cell/transfusion). A week later, serum samples were collected and assayed for anti–H-2\textsuperscript{d} antibody activities by immunofluorescence flow cytometry. The mean fluorescent intensity of pooled preimmune serum was 30.

treated with 0 to 4 weekly transfusions of UVB-irradiated leukocytes. As shown in Table 3, although 30% of recipient mice became tolerant after 1 transfusion with UVB-irradiated leukocytes, a minimum of 4 weekly transfusions were necessary to ensure that all treated mice became tolerant to donor MHC antigens. Based on these results, 4 weekly transfusions of UVB-irradiated peripheral blood MNLs were routinely used for tolerance induction, and humoral immune tolerance to donor MHC antigens has been reproducibly induced in all recipient mice studied (n = 200).

**Durability of the induced immune tolerance.** To determine the durability of the induced tolerance, immune tolerance to H-2\textsuperscript{d} MHC antigens was induced in 5 CBA mice. These 5 mice were challenged periodically with a fully immunogenic dose of donor MNLs (2 \times 10^7 cells/transfusion over a period of 35 weeks). For each series of transfusion challenge, 2 age-matched naïve CBA mice were included as controls to ensure that the transfused donor leukocytes were immunogenic. As shown in Fig 2, none of the tolerant CBA mice became alloimmunized after repeated challenges with untreated BALB/c mice MNLs. In contrast, all naïve control recipient mice became immunized. In a separate experiment, 5 tolerant CBA mice were challenged with 2 transfusions of untreated donor leukocytes 12 weeks after the last transfusion of UVB-irradiated leukocytes, and all 5 mice remained tolerant. These results indicate that the induced tolerance can last for at least 12 weeks and can withstand repeated immunological challenges.

**Effect of tolerance induction on humoral immune responses.** To determine whether the induced tolerance has any nonspecific inhibitory effect on humoral immune responses to antigens unrelated to MHC molecules and donor leukocytes.

**MHC specificity of the induced tolerance.** To determine whether the induced tolerance included MHC antigens other than the donor H-2\textsuperscript{d} phenotype, tolerant CBA mice were challenged with peripheral blood MNLs carrying H-2 antigens different from those of the original donor BALB/c mice. The three different strains of mice used are not closely related to BALB/c. As shown in Table 4, H-2\textsuperscript{d}–tolerant CBA mice also became tolerant to H-2\textsuperscript{b} and H-2\textsuperscript{e} antigens of SJL mice, the antibody activities in the tolerant CBA mice were significantly lower than those of the control CBA mice (Table 4). These results indicate that the tolerance induced by UVB-irradiated leukocytes is not restricted to donor H-2\textsuperscript{d} antigens. This finding is consistent with earlier observation made in a canine transfusion model.

**Adoptive transfer of immune tolerance.** The result of a previous study suggested that transfusions of UVB-irradiated leukocytes may induce the development of negative regulatory cells, causing the antibody response to subsequent challenge with untreated donor leukocytes to be attenuated. To further investigate this possibility, adoptive transfer of immune tolerance by spleen MNLs from tolerant mice to naïve mice was conducted. Each naïve CBA mouse was infused with 3 \times 10^7 spleen MNLs from tolerant CBA mice. Beginning 3 days after the adoptive transfer, each mouse received the first of 4 weekly challenges with 4 \times 10^7 untreated BALB/c mice MNLs. The results of two separate experiments show that the antibody responses against donor MHC antigens were significantly attenuated in mice receiving spleen MNLs from the tolerant CBA mice (Fig 4).

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**Table 3. Dose Dependency of Tolerance Induction by UVB-Irradiated Donor Leukocytes**

<table>
<thead>
<tr>
<th>No. of Weekly Transfusions With UVB-WBC</th>
<th>No. of Recipient CBA Mice</th>
<th>Anti–H-2\textsuperscript{d} Antibody Activity (mean fluorescent intensity*)</th>
<th>Mice Tolerant to H-2\textsuperscript{d}</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>7</td>
<td>271, 319, 396, 394, 238, 266, 114</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>1</td>
<td>7</td>
<td>82, 73, 367, 64, 46, 32, 33</td>
<td>2 (28%)</td>
</tr>
<tr>
<td>2</td>
<td>7</td>
<td>52, 75, 133, 26, 29, 31, 32</td>
<td>4 (57%)</td>
</tr>
<tr>
<td>3</td>
<td>7</td>
<td>72, 73, 123, 30, 27, 29, 32</td>
<td>4 (57%)</td>
</tr>
<tr>
<td>4</td>
<td>7</td>
<td>29, 33, 24, 34, 28, 32, 30</td>
<td>7 (100%)</td>
</tr>
</tbody>
</table>

* One week after the last transfusion of UVB-irradiated leukocytes, each mouse was challenged with 2 weekly transfusions of untreated BALB/c donor leukocytes (2 \times 10^5 cells/transfusion). A week later, serum samples were collected and assayed for anti–H-2\textsuperscript{d} antibody activities by immunofluorescence flow cytometry. The mean fluorescent intensity of pooled preimmune serum was 30.

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**Fig 2. Durability of humoral immune tolerance induced by transfusions of UVB-irradiated BALB/c donor leukocytes.** (A) 5 CBA mice received 4 weekly transfusions of UVB-irradiated donor leukocytes (•); (B) 5 control CBA mice received 4 weekly transfusions of untreated donor leukocytes (○); (C) naïve control CBA mice (n = 2) treated with the same unirradiated donor leukocytes for each series of 4 or 2 weekly transfusion challenges (□).
UVB-WBC TRANSFUSION AND TOLERANCE INDUCTION

Effect of transusions of UVB-irradiated leukocytes on prior alloimmunity to donor MHC antigens. To ascertain whether preexisting alloimmunization to donor MHC antigens could be attenuated by transusions of UVB-irradiated donor MLNs, CBA mice were alloimmunized against BALB/c mice H-2\textsuperscript{d} antigens. The immunized CBA mice were then randomly assigned into two groups. Group A was treated with 4 weekly transusions of UVB-irradiated donor peripheral blood MLNs (2 x 10\textsuperscript{5} MLNs/transfusion.) Group B did not receive any further transusions. Serum samples were collected before, during, and after transusions with UVB-irradiated leukocytes. Antibody activities measured by immunofluorescence flow cytometry showed that transusions of UVB-irradiated leukocytes were unable to suppress a preexisting antibody response to donor MHC antigens (Fig 5A). In contrast, antibody activities in the control group B continued to decrease over the time course of the experiment (Fig 5B). Therefore, UVB-irradiated MLNs actually appeared to be able to maintain the antibody activities in mice with prior alloimmunization to donor H-2\textsuperscript{d} antigens. This finding indicates that transusion of UVB-irradiated leukocytes is not effective to suppress the existing immunity and can be used to induce tolerance only in recipients without prior immunization to donor MHC antigens.

DISCUSSION

The results of this study indicate that the elimination of plasma and platelets from UVB-irradiated donor leukocytes is essential for the successful induction of humoral immune tolerance to donor MHC antigens. Although the exact mechanism of tolerance induction by UVB-irradiated leukocytes has yet to be elucidated, studies using 8-methoxypsoralene and irradiation of long wavelength UV light (UVA; 320 to 400 nm) to cross-link DNA and inactivate mouse donor leukocytes showed that such treatment is effective in reducing the alloantigenicity of platelet concentrates. This suggested that stimulatory factors, not constitutively expressed by donor leukocytes, need to be synthesized and expressed to alloimmunize transfusion recipients. The results of further studies have shown that donor leukocytes positive for class-II MHC antigens are responsible for alloimmunizing mouse transfusion recipients to donor MHC antigens. Both B7-1 (CD80) and B7-2 (CD86) molecules, expressed by activated class-II MHC antigen-positive leukocytes, are potent costimulatory signals to helper T cells\textsuperscript{35} and play crucial roles in initiating humoral and cellular immune responses in hosts. All these findings suggest that the alloimmunization of transfusion recipients by donor leukocytes could entail the following sequence of events.

After transusion of blood products, class-II MHC antigen-positive donor leukocytes, which include B cells, monocytes, and dendritic cells, interact with alloreactive helper T cells of recipients. This direct interaction between donor class-II MHC molecules and recipient T-cell receptors leads to activation of donor leukocytes and expression of CD80 and/or CD86 costimulatory signals. Recipient helper T cells then are further activated by CD80 and/or CD86. The activation of recipient helper T cells results in alloimmunization of transfusion recipients to donor MHC antigens. Because UVB irradiation is known to inhibit the expression of interleukin-1, CD80, and CD86 by monocytes activated with lipopolysaccharide or T cells,\textsuperscript{32,33} the suppressed expression of these costimulatory signals may prevent the full activation of recipient T helper cells. Consequently, T cells are anergized and immune tolerance ensues.

In addition to this direct antigen presentation pathway, large amounts of donor MHC antigens on platelets and in plasma can be processed and presented indirectly by antigen-presenting cells of transfusion recipients to their own immune systems. This indirect pathway of antigen presentation could also lead to alloimmunization.\textsuperscript{8,34} Because class-I MHC molecules per se are poorly immunogenic, it is likely that the indirect presentation pathway is not a major route for alloimmunizing transfusion recipients to donor MHC antigens. For this reason, larger quantities of class-I MHC antigens may be required to immunize transfusion recipients through the indirect pathway.

As reported previously, more than 90% of class-I MHC antigens in human whole blood are present on platelets and in plasma.\textsuperscript{35} Thus, transfusion recipients of platelet concentrates are routinely exposed to large amounts of donor class-I MHC antigens. Indirect presentation of donor class-I MHC antigens...
Table 4. Challenges of CBA Mice Tolerant to BALB/c MHC Antigens With Leukocytes From Three Different Inbred Strains of Mice

<table>
<thead>
<tr>
<th>Recipient CBA Mouse*</th>
<th>No.</th>
<th>Mouse Strain of Challenging Leukocytes</th>
<th>H-2 Phenotype</th>
<th>Incidence of Alloimmunization (%)</th>
<th>Antibodies to Challenging Leukocytes†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Naive</td>
<td>5</td>
<td>BALB/c</td>
<td>d</td>
<td>100</td>
<td>234 ± 29</td>
</tr>
<tr>
<td>Tolerant</td>
<td>5</td>
<td>BALB/c</td>
<td>d</td>
<td>0</td>
<td>32 ± 7</td>
</tr>
<tr>
<td>Naive</td>
<td>5</td>
<td>C57BL/6</td>
<td>b</td>
<td>100</td>
<td>103 ± 10</td>
</tr>
<tr>
<td>Tolerant</td>
<td>5</td>
<td>C57BL/6</td>
<td>b</td>
<td>0</td>
<td>21 ± 4</td>
</tr>
<tr>
<td>Naive</td>
<td>5</td>
<td>RII</td>
<td>r</td>
<td>100</td>
<td>123 ± 31</td>
</tr>
<tr>
<td>Tolerant</td>
<td>5</td>
<td>RII</td>
<td>r</td>
<td>0</td>
<td>13 ± 4</td>
</tr>
<tr>
<td>Naive</td>
<td>5</td>
<td>SJL</td>
<td>s</td>
<td>100</td>
<td>464 ± 61</td>
</tr>
<tr>
<td>Tolerant</td>
<td>5</td>
<td>SJL</td>
<td>s</td>
<td>100</td>
<td>74 ± 20</td>
</tr>
</tbody>
</table>

* Naive and tolerant CBA mice were challenged with 2 weekly transfusions of untreated peripheral blood MNLs from four inbred strains of mice carrying different H-2 antigens as shown above.
† Antibodies were assayed by immunofluorescence flow cytometry using spleen leukocytes of challenging mice as targets. Each value is mean ± SD.
‡ Mean fluorescent intensity of preimmune sera collected from naive CBA mice.

Antigens may compete with induction of anergy by donor leukocytes in UVB-irradiated platelet concentrates and interfere with tolerance induction. This could explain the inconsistent or partial induction of immune tolerance by transfusions of UV-irradiated platelet concentrates in earlier animal studies and may be responsible for the recently reported failure of UVB-irradiated platelet concentrates to prevent HLA alloimmunization in patients undergoing cardiopulmonary bypass surgery.

To test whether indirect antigen presentation interferes the induction of humoral immune tolerance, the effect of platelets and plasma on tolerance induction by UVB-irradiated donor leukocytes was investigated in a mouse transfusion model. To determine whether mouse platelets, similar to their human counterparts, also express significant amounts of class-I MHC antigens, the number of class-I MHC molecules on platelets of donor BALB/c mice were measured using 125I-labeled Fab fragments of anti-H-2d MoAbs and Scatchard analysis as described previously. An average 1 × 105 class-I MHC molecules were found on each BALB/c mouse platelet (data not shown). Although this number is considerably less than that found on a human platelet (1,000 × 80,000 molecules, respectively), the total amount of platelet class-I MHC antigens transfused into each recipient mouse (6.3 × 107 platelets/transfusion) was significant. The transfused murine platelets contain more than 2 times the number of class-I MHC antigen molecules as do MNLs. Thus, a significant number of class-I MHC antigens on mouse platelets may interfere with tolerance induction. Another potential source of MHC antigens that may interfere with tolerance induction is shedding of MHC antigens from leukocytes after UVB-irradiation. However, quantitative reduction of class-II MHC antigens on leukocytes after UVB-irradiation is minimal. Therefore, shedding of MHC antigens is not likely to have significant impact on tolerance induction by UVB-irradiated leukocytes as shown in Fig 1.
UVB-WBC TRANSFUSION AND TOLERANCE INDUCTION

CBA mice produced humoral immune responses to challenges by leukocytes from H-2^d-positive SJL mice (Table 4) provides further evidence for the development of negative regulatory cells in tolerant CBA mice.

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REFERENCES


To further elucidate the cellular basis of induced tolerance, adoptive transfer experiments were performed (Fig 4). Although spleen MNLs of tolerant CBA mice were unable to transfer complete humoral immune tolerance to naive CBA mice, naive mice receiving spleen leukocytes from the tolerant mice did show significantly decreased humoral immune responses to challenges with untreated BALB/c donor leukocytes (Fig 4). In these studies, a submaximal antigenic dose of untreated donor leukocytes (4 x 10^9 cells) was used to increase the sensitivity for detecting any presence of inhibitory activity. These findings suggest that the development of negative regulatory cells may be partially responsible for the suppression of humoral immune responses to donor MHC antigens in tolerant mice. The studies also show that the adoptive transfer system can be used to further identify which subset(s) of MNLs is responsible for the observed inhibitory activity and to elucidate the cellular mechanism responsible for the tolerance induced by UVB-irradiated donor leukocytes. In addition, the finding that the CBA mice tolerant to BALB/c H-2^d antigens developed reduced humoral immune responses to challenges by leukocytes from H-2^d-positive SJL mice (Table 4) provides further evidence for the development of negative regulatory cells in tolerant CBA mice.

Fig 5. Effect of UVB-irradiated donor leukocytes on prior alloimmunity to donor MHC antigens. (A) Five CBA mice alloimmunized to BALB/c mice H-2^k-^d antigens (△) and three naive CBA mice (◇) were treated with 4 transfusions of UVB-irradiated BALB/c leukocytes (□). (B) The other 4 alloimmunized CBA mice (◇) were not transfused with any UVB-irradiated leukocytes.

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