Prevention of Graft-Versus-Host Disease by Induction of Immune Tolerance With Ultraviolet B-Irradiated Leukocytes in H-2 Disparate Bone Marrow Donor

By M.L.U. del Rosario, James R. Zucali, and K.J. Kao

In spite of the widespread use of allogeneic bone marrow transplant (BMT) in the treatment of various malignant and nonmalignant conditions, graft-versus-host disease (GVHD) continues to be a serious complication after allogeneic BMT. GVHD, whether acute or chronic, has been shown to occur in 30% to 60% of patients receiving histocompatible sibling-matched allogeneic BMT.1 Mortality attributed to GVHD has been shown to occur in up to 50% of cases.2-4 The severity of GVHD is related to the degree of difference between hosts and donors across the major histocompatibility (MHC) barrier5,6 and the presence of donor T cells in marrow grafts.7-10 For this reason, T-cell depletion from marrow grafts has been successfully applied to prevent GVHD. Unfortunately, this approach has been associated with increased incidences of graft failure, more severe immunosuppression, and higher relapse rates of original malignancies.7,8,11-14 Therefore, developing an approach to prevent or attenuate GVHD.

Irradiation of leukocytes with medium (UVB) or short (UVC) wavelength ultraviolet light has been shown to produce various immunomodulatory effects.15-21 These effects include inhibition of stimulator and responder function of leukocytes in mixed leukocyte culture (MLC),15,16 suppression of cytokine production,17 downregulation of cell membrane proteins such as class-II MHC antigens18 and cell adhesion molecules,19 reduced lymphocyte proliferative responses to mitogenic lectins,20 and inhibition of expression of costimulatory signals by antigen presenting cells.21 On the basis of these findings, UVC and UVB have been applied to inactivate leukocytes for reducing the immunogenicity of platelet concentrates.22-25 Results from these studies indicate that transfusions of platelet concentrates irradiated with UVB or UVC could induce humoral immune tolerance to allogeneic MHC antigens. However, the induced tolerance was either incomplete22 or inconsistent.24

To overcome the problems of inconsistency and partial tolerance, we investigated the effect of plasma and platelets on tolerance induction by UVB-irradiated leukocytes. Our study showed that the presence of plasma and platelets interfered with tolerance induction and that the use of highly purified peripheral mononuclear leukocytes (MLN) is critical for consistent induction of complete humoral immune tolerance to allogeneic MHC antigens.26 However, it is not known whether cellular immune tolerance can be induced by the same approach. To answer this question and to explore the potential application of this approach to allogeneic BMT, we conducted experiments to determine whether transplantation with bone marrow and spleen cells from H-2 disparate tolerant donor mice could prevent or attenuate the GVHD induced by allogeneic BMT in a murine model.

MATERIALS AND METHODS

Animals. Eight-week old CBA/CaH-T6( CBA) mice with H-2K haplotype and BALB/cByJ (BALB/c) mice with H-2D haplotype were obtained from Jackson Laboratory (Bar Harbor, ME). BALB/c mice were also obtained from Harlan Sprague Dawley, Inc (Indianapolis, IN). All mice were housed in a temperature-controlled room (25°C) with a 12-hour interval light/dark cycle and fed ad libitum.

© 1999 by The American Society of Hematology.
Immune tolerance and bone marrow transplant

Preparation of UVB-irradiated peripheral mononuclear leukocytes. Peripheral mononuclear leukocytes were prepared from freshly collected BALB/c venous blood by differential and Ficoll-hypaque density gradient centrifugation as described. MNL were suspended in phosphate buffered saline (PBS) and their concentrations were determined by hemacytometers after staining cells with propidium iodide solution. For irradiation of leukocytes by UVB, a Bioslink-UV irradiator (BIOS Corp, New Haven, CT) with a built-in dosimeter was used. The dose of UVB irradiation was 1.200 mL/cm². The irradiation was performed in an open sterile polypolypropylene container. The depth of cell suspension during UVB-irradiation was 1 mm. The cell suspensions were mixed manually by moving the tray back and forth during irradiation.

Induction of immune tolerance. Immune tolerance was induced in CBA mice by four weekly intravenous injections of 2 × 10⁶ UVB-irradiated BALB/c MNL in 100 µL PBS through a tail vein under light anesthesia with inhalation of methoxyflurane (Pitman-Moore Inc, Mundelein, IL). Preimmune serum samples were prepared from venous blood that was collected by retro-orbital bleeding 2 days before the first transfusion. One week after the last weekly transfusion of UVB-irradiated leukocytes, serum samples were collected to assess the development of antibodies to donor MHC antigens by flow cytometry as described. As reported previously, CBA mice became tolerant, if they did not develop any anti-H2d antibodies after four weekly transfusions of UVB-irradiated BALB/c MNL. To insure the development of tolerance, two transfused CBA mice were randomly selected and challenged with two weekly transfusions of 1 × 10⁶ untreated BALB/c MNL. We found that none of the challenged CBA mice became immunized to H-2k antigens of BALB/c mice as expected. In contrast, all control naïve CBA mice developed anti-H-2d antibody after two transfusion challenges.

BMT. Naïve and tolerant CBA mice were used as donors of bone marrow and spleen cells. BALB/c mice were used as bone marrow recipients. BMC from CBA mice were prepared by flushing cells out of femurs and tibias using 25-gauge needles and syringes filled with RPMI 1640 medium as described. Spleen MNL were prepared from bone marrow donor mice by Ficoll gradient centrifugation. Fifty µL of spleen MNL containing specified numbers of cells were mixed with 5 × 10⁶ bone marrow cells in a final volume of 200 µL. These cells were then injected through a tail vein into each BALB/c mouse that had been lethally irradiated with 750 cGy gamma ray 5 to 6 hours before transplantation. The transplanted mice were fed with sterilized laboratory chow and acidified water. They were followed for engraftment of donor cells by immunofluorescence flow cytometry. Body weight, peripheral total and differential white cell count, GVHD, and survival were measured.

Immunofluorescence flow cytometry for detection of engraftment and characterization of lymphocyte subsets. H-2 phenotypes of peripheral blood leukocytes were determined by immunofluorescence flow cytometry using fluorescein isothiocyanate (FITC)-labeled anti-H2d antibody and phycoerythrin (PE)-labeled anti-H2k antibody. Different subsets of lymphocytes including B cells, CD4+ helper T cells, and CD8+ cytotoxic T cells were measured using PE-labeled anti-B220, FITC-labeled anti-CD4, and PE-labeled anti-CD8 antibodies. All antibodies were purchased from PharMingen Corp (San Diego, CA). Peripheral leukocytes for flow-cytometric analysis were prepared by washing 20µL of whole blood with 1 mL PBS and lysing red cells with 1.5 mL of 0.85% ammonium chloride solution. After lysis of red cells, the remaining cells were washed twice with 0.5 mL PBS and resuspended in 100 µL PBS-0.02% azide-0.5% bovine serum albumin (BSA). Fifty µL of washed leukocytes were stained with appropriate concentrations of fluorescent antibodies for 30 minutes. After two washes, the cells were analyzed using a FACScan flow cytometer (Becton Dickinson, San Jose, CA).

Enzyme-linked immunoassays (ELA) for interleukin-4 (IL-4) and γ-interferon (γ-IFN). For the assays, plastic wells of a microtiter plate were coated with 50 µL of 5µg/mL antimouse IL-4 or γ-IFN antibody in PBS-azide overnight at 4°C. The plates were washed three times using PBS-azide-0.2%. Tween-20 and blocked with 150 µL PBS containing 10% newborn calf serum (NCS) for 30 minutes at room temperature. Thereafter, 50 µL of cytokine standards and diluted serum samples were added and incubated overnight at 4°C. Serum samples were diluted with equal volume of PBS-10% NCS. After overnight incubation, plates were washed and incubated sequentially with 50 µL of 5 µg/mL biotinylated anticytokine antibody diluted in PBS-Tween-1% BSA, strepavidin-peroxidase (Sigma Co, St Louis, MO), and o-phenylenediamine dihydrochloride peroxidase substrate (Sigma Co). Paired anti-IL-4 and anti-γ-IFN antibodies, recombiant murine IL-4 and γ-IFN used in the assays were obtained from PharMingen (San Diego, CA) and Endogen (Woburn, MA), respectively. The lowest concentrations of IL-4 and γ-IFN that can be measured by these two assays were 0.2 pg/mL, and 50 pg/mL, respectively.

GVHD scoring. A scoring system was devised to grade GVHD in the transplanted mice. Hair and skin changes involving the head/neck, abdomen and tail, and changes in anal and perianal mucosa were scored according to severity from 0 to 3 as defined in Table 1. Mice with a total score of less than 3 were regarded as having mild GVHD, ≥3 to ≤5 as having moderate GVHD and total score of greater than 5 as severe GVHD.

RESULTS

Induction of GVHD. Unlike humans, only a small number of T cells are present in murine bone marrow. According to our flow cytometric study, CD3+ T cells account for less than 1% of total bone marrow cells harvested from CBA mice. Therefore, transplantation of allogeneic bone marrow cells alone does not always result in clinically apparent GVHD in BALB/c recipient mice. Addition of appropriate numbers of spleen MNL to BMC before transplantation is necessary to induce clinically apparent GVHD. To determine the optimal numbers of spleen MNL that are required for inducing clinically significant GVHD, we first studied how different numbers of spleen MNL mixed with 5 × 10⁶ BMC affected the severity of GVHD. The results of our study indicate that moderate to severe GVHD can be induced by

---

**Table 1. Grading Criteria for GVHD in Transplanted Mice**

<table>
<thead>
<tr>
<th>Criteria</th>
<th>Grade 0</th>
<th>Grade 1</th>
<th>Grade 2</th>
<th>Grade 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hair loss, neck and head</td>
<td>Normal</td>
<td>Normal</td>
<td>Multi-patchy hair loss</td>
<td>Multi-patchy hair loss</td>
</tr>
<tr>
<td>Hair loss, chest &amp; abdomen</td>
<td>Normal</td>
<td>Normal</td>
<td>Multiple stripes</td>
<td>Multiple stripes</td>
</tr>
<tr>
<td>Area involved</td>
<td>Normal</td>
<td>Normal</td>
<td>≥20%, &lt;50%</td>
<td>≥20%, &lt;50%</td>
</tr>
<tr>
<td>Skin integrity tail</td>
<td>Normal</td>
<td>Normal</td>
<td>Hair loss, patchy-hyperkeratosis</td>
<td>Hair loss, patchy-hyperkeratosis</td>
</tr>
<tr>
<td>Perianal and anal mucosa</td>
<td>Normal</td>
<td>Normal</td>
<td>Hair loss, diffuse-hyperkeratosis</td>
<td>Hair loss, diffuse-hyperkeratosis</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Areas of desquamation</td>
<td>Areas of desquamation</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Inflammation and prolapsoe of anal mucosa</td>
<td>Inflammation and prolapsoe of anal mucosa</td>
</tr>
</tbody>
</table>

---
including 2.5 to 4 × 10⁵ donor spleen MNL with 5 × 10⁶ BMC. Transplantation of 1 × 10⁶ or more spleen MNL was associated with hyperacute GVHD in which most recipient BALB/c mice died within 8 days after transplantation. Inclusion of less than 1 × 10⁶ spleen MNL was associated with nil to mild GVHD. GVHD was judged by the presence of hair loss, skin changes, body weight loss, and stunted weight gain and scored according to the criteria described in Table 1.

**GVHD after transplantation with bone marrow and spleen cells from tolerant donors.** To determine whether GVHD can be prevented or attenuated by transplantation of cells from CBA mice tolerant to MHC antigens of BALB/c mice, we studied how the addition of 4 × 10⁵ or 2.5 × 10⁵ spleen MNL from naive or tolerant CBA mice to their respective bone marrow cells affected the transplantation associated GVHD in recipient BALB/c mice. All transplanted BALB/c mice were followed weekly for changes in body weight and development of GVHD. As shown in Fig 1 and 2, recipient BALB/c mice transplanted with two different doses of naive control spleen cells and 5 × 10⁶ BMC had poor body weight gain and moderate to severe GVHD. Most BALB/c mice transplanted with two different doses of tolerant spleen MNL and 5 × 10⁶ BMC had better body weight recovery (Fig 1) and significantly reduced GVHD (Fig 2) when they were compared with recipients of naive control cells.

**Engraftment and recovery of peripheral leukocytes.** Two weeks after BMT, all H-2d recipient mice became completely engrafted with donor H-2k leukocytes in peripheral blood as assessed by immunofluorescence flow cytometry. To determine whether there was any difference in the rate of engraftment, peripheral total and differential leukocyte counts were followed. Peripheral lymphocyte counts including T and B cell subsets were measured according to peripheral blood total and differential leukocyte counts and immunophenotyping results for T and B cells by flow cytometry. As shown in Fig 3, there was no significant difference in the initial recovery of peripheral leukocyte counts between BALB/c recipient mice transplanted with either naive or tolerant donor bone marrow and spleen cells. Nevertheless, lymphocyte recovery was faster especially in recipients of 4 × 10⁵ tolerant spleen MNL (Fig 4, Experiment II). BALB/c mice transplanted with 5 × 10⁶ BMC and 4 × 10⁵ spleen MNL from tolerant donors also showed a faster and more complete recovery of T and B cells in the peripheral blood (Fig 5). In addition, it was noted that the recovery of CD8+ T cells was also faster after transplantation with bone marrow and spleen cells from tolerant donor mice (Fig 6). As shown in Fig 6, the recovery of CD8+ T cells in general lagged behind CD4+ T cells in our murine bone marrow transplantation model.
Survival after BMT. Because the initial results of our study (Fig 1) showed that transplantation of tolerant BMC and spleen MNL had a significant positive effect on overall survival, two additional bone marrow transplantation experiments using $5 \times 10^6$ BMC and $4 \times 10^5$ spleen MNL were conducted to confirm this finding. There were five mice in each experimental group. The survival data from these three separate experiments were pooled and analyzed. As shown in Fig 7, the overall long-term survival of mice transplanted with cells from tolerant donors was 72% versus 17% ($P = .018$) for those transplanted with naive cells.

**Fig 3.** Changes of WBC counts in peripheral blood after transplantation of lethally irradiated BALB/c mice with bone marrow and spleen cells from naive (●,▲) or tolerant (□,△) CBA mice as described in Fig 1. Experiment I: $5 \times 10^6$ bone marrow cells + $2.5 \times 10^5$ spleen MNL. Experiment II: $5 \times 10^6$ bone marrow cells + $4 \times 10^5$ spleen MNL. Each value is mean ± SD.

**Fig 4.** Changes of lymphocyte counts in peripheral blood after transplantation of lethally irradiated BALB/c mice with bone marrow and spleen cells from naive (●,▲) or tolerant (□,△) CBA mice as described in Fig 1. Experiment I: $5 \times 10^6$ bone marrow cells + $2.5 \times 10^5$ spleen MNL. Experiment II: $5 \times 10^6$ bone marrow cells + $4 \times 10^5$ spleen MNL. Each value is mean ± SD.

**Fig 5.** Changes of B and T cell counts in peripheral blood after transplantation of lethally irradiated BALB/c mice with $5 \times 10^6$ bone marrow and $4 \times 10^5$ spleen cells from naive (▲) or tolerant (△) CBA mice. Each value is mean ± SD.

**Fig 6.** Changes of CD4/CD8 T cell ratio in peripheral blood after transplantation of lethally irradiated BALB/c mice with $5 \times 10^6$ bone marrow and $4 \times 10^5$ spleen cells from naive (▲) or tolerant (△) CBA mice. Each value is mean ± SD.
T-cell cytokine response after BMT. According to our recent study,29 tolerance induction by transfusions with UVB-irradiated BALB/c leukocytes polarizes T cells of CBA mice to produce of type-2 cytokines during in vitro stimulation by BALB/c spleen cells. It was of interest to study the serum levels of $\gamma$-IFN and IL-4 in BALB/c mice after transplantation with cells from tolerant CBA donors. In this study, three mice from each treatment group were randomly selected and sacrificed every other day until day 9 after BMT. Equal volumes of serum samples from three mice were pooled to obtain sufficient amounts of samples for assays of IL-4 and $\gamma$-IFN. The results show that recipient mice transplanted with tolerant BMC and spleen MNL had lower peak serum levels of $\gamma$-IFN and higher levels of IL-4 than those transplanted with cells from naive CBA donor mice after BMT, respectively (Fig 8).

DISCUSSION

T cells in allogeneic bone marrow are responsible for BMT-associated GVHD.7-10 Therefore, rendering donor T cells tolerant toward recipient MHC antigens offers an attractive approach to prevent or reduce GVHD. Our recent study showed that humoral immune tolerance to allogeneic MHC antigens can be induced without nonspecific immunosuppression by transfusions of UVB-irradiated leukocytes from H-2 disparate mice.26 It was therefore of interest to learn whether GVHD can be prevented by transplantation of bone marrow and spleen mononuclear cells from H-2 disparate donor mice that have been tolerized toward MHC antigens of BMT recipient mice. To answer this question, we conducted such a study. The results reported herein show that transplantation with bone marrow and spleen cells from tolerant CBA (H2k) donor mice to BALB/c (H2d) recipient mice is associated with significant attenuation of GVHD, better long-term survival, faster reconstitution of peripheral lymphocytes, and Th2 cytokine response immediately after transplantation. Because cell-mediated immunity plays an important role in the pathogenesis of GVHD, these findings suggest that transfusions of UVB-irradiated leukocytes may induce cellular immune tolerance. This conclusion is further substantiated by our recent study of cytotoxic T-cell activity against H-2d$^+$ target cells in the CBA mice that had been tolerized by transfusions of UVB-irradiated BALB/c mononuclear leukocytes and subsequently challenged with two fully immunogenic doses of untreated BALB/c leukocytes (unpublished observation).

In our study, two different doses of spleen MNL (2.5 $\times$ 10$^5$ and 4 $\times$ 10$^5$) were used to induce GVHD. These two doses were selected for their ability to elicit significant degrees of acute GVHD without causing premature death so that the transplanted mice could be followed for an extended period of time. Among BALB/c recipient mice transplanted with cells from naive donor mice, we noted that recipient BALB/c mice transplanted with a lower number (2.5 $\times$ 10$^5$) spleen MNL had less severe and lasting GVHD (Fig 2). This finding confirms the quantitative importance of donor lymphocytes in eliciting GVHD as previously reported.30-33 The results of our study also showed that 1 month after BMT, peripheral leukocyte counts recovered to normal levels in most recipient mice regardless of whether donor cells were from naive or tolerant CBA mice. However, during the next 4 months peripheral leukocyte counts began to decline until GVHD largely resolved 150 days after BMT. In addition to GVHD, this decline of peripheral leukocyte counts was likely a result of delayed repopulation by more primitive
long-term repopulating hematopoietic stem cells as observed in bone marrow transplant recipients. We also noted that recovery of peripheral T- and B-lymphocyte counts was faster in mice transplanted with bone marrow and spleen cells from the tolerant CBA donors (Fig 5). The cell counts and immunophenotyping results are consistent with the subjective clinical assessment of GVHD. All these findings support the beneficial effect of using cells from the tolerant donor mice for bone marrow transplantation across major MHC barrier. However, it is not known whether faster quantitative lymphoid reconstitution would translate to better immune function. Further studies in this regard are needed.

According to cytokine production profile, two subsets of helper (Th) and cytotoxic (Tc) T cells have been identified. Secretion of γ-IFN and tumor necrosis factor-β (TNF-β) defines type-1 Th cells (Th1), and secretion of IL-4, IL-5, and IL-10 defines type-2 Th cells (Th2). Th1 cells are functionally involved in cell-mediated immunity, delayed-type hypersensitivity, production of IgG2a and IgG2b antibodies, and host defense to infection of intracellular pathogens. Th2 cells are involved in production of IgG1 and IgE antibodies, allergic conditions, and host defense to infection of extracellular pathogens. Th1 and Th2 responses during host-immune response have been shown to be mutually inhibitory. Thus, immune responses can be characterized by polarization toward Th1 or Th2 response.

Induction of tolerance by priming neonatal mice with allogeneic leukocytes has been associated with increased production of Th2 cytokines and reduced secretion of Th1 cytokines. The finding indicates that tolerance induction leads the immune system towards an enhanced Th2 response. Our recent study of cytokine production in mixed leukocyte culture reactions using tolerized CBA spleen T lymphocytes as responder and BALB/c spleen leukocytes as stimulators showed an increase in IL4 and IL5 production (data not shown). In allogeneic BMT, donor lymphocytes with enhanced Th2 cytokine response have been associated with a much-reduced acute GVHD. For this reason, we measured serum IL-4 and γ-IFN levels after transplantation with bone marrow and spleen cells from tolerant or naive donor CBA mice. Our results showed an enhanced transient Th2 cytokine response in recipient mice transplanted with cells from tolerant donors. The results are consistent with our recent in vitro MLC finding and support the earlier reports that polarization of donor T cells to Th2 response is associated with reduced severity of acute GVHD. Nevertheless, we are unable to ascertain whether the observed differences in cytokine production were due to donor cells, recipient cells, or both. Although the polarized Th2 response in donors may contribute to protection of acute GVHD as suggested by various investigators, it is possible that donor Th2 cytokine response only reflects changes in the effector arm of the donor immune system and may not play a direct role in the prevention of GVHD associated with allogeneic BMT. The exact mechanism by which Th2 polarization of the bone marrow donor immune system could attenuate acute GVHD remains to be further elucidated.

In the present study, we found that acute GVHD can not be completely prevented by transplantation of bone marrow and spleen cells from tolerant CBA donors (Fig 2). This finding is not unexpected. Our recent MLC study using T cells from tolerant CBA mice as responders and gamma-irradiated or mitomycin-treated spleen leukocytes from BALB/c mice as stimulators indicated that T cells from tolerant CBA mice remain capable of producing type-1 T-cell cytokines such as γ-IFN but at a reduced level. Because type-1 T-cell cytokines have been implicated in the pathogenesis of acute GVHD, the reduced production of type-1 T-cell cytokines by T cells from tolerant donor mice might be responsible for the attenuated GVHD observed in our experiments.

In view of the feasibility and the safety of transfusing patients with UVB-irradiated platelet concentrates, the results of our study support the potential clinical application of UVB-irradiated leukocytes for tolerance induction in bone marrow donors to prevent GVHD. However, the safety and the ethical concerns of using leukocytes from patients with neoplastic diseases to induce tolerance in healthy bone marrow donors preclude such an approach. Nevertheless, tolerance induction in related bone marrow donors may be considered for patients who suffer from hereditary non-neoplastic hematological disorders, such as sickle cell anemia and thalassemia and who are not at risk of transmitting infectious diseases to their bone marrow donors. Another potential approach is to induce immune tolerance in patients using UVB-irradiated leukocytes from healthy bone marrow donors. The tolerated patients would then undergo bone marrow transplant using a nonmyeloablative conditioning regimen. Such an approach may allow the establishment of macro-mixed chimerism that is sufficient to ameliorate non-neoplastic hematological disorders without increasing the risk of graft rejection. At the same time, high-dose chemoradiotherapy related toxicity is avoided. Obviously, further animal studies are needed before the proposed approach can be applied to the clinical setting.

ACKNOWLEDGMENT

The authors are grateful to Sandra Donahue for her excellent technical assistance.

REFERENCES

host disease in a murine bone marrow transplanted model, in Barakoff
SJ, Deeg HJ, Ferrara J, Atkinson K (eds): Graft versus Host Disease,
New York, NY, Marcel Dekker, 1990, p 9
10. Deeg HJ, Cotter-Fox M: Clinical spectrum and pathophysiology
of acute graft-vs.-host disease, in Barakoff SJ, Deeg HJ, Ferrara J,
Atkinson K (eds): Graft-versus-Host Disease, New York, NY, Marcel
Dekker, 1990, p 311
11. Champlin RE: T-cell depletion for bone marrow transplantation:
Effects on graft rejection, graft-versus-host disease, graft-versus-
12. Delain M, Cahn JY, Racadot E, Flesch M, Plouvier E, Mercier
M, Tiberghien P, Pavy JJ, Deschaseaux M, Deconinck E: Graft failure
after T-cell depleted HLA identical allogeneic bone marrow transplan-
transplantation: Risk factors in leukemia patients. Leuk Lymphoma
11:359, 1993
13. Pirsch JP, Mahi DG: Infectious complications in adults with bone
marrow transplantation and T-cell depletion of donor marrow. Increased
14. Goldman JM, Gale RP, Horowitz MM, Biggs JC, Champlin RE,
Gluckman E, Hoffman RG, Jacobsd SJ, Marmont AM, McGlave PB:
Bone marrow transplantation for chronic myelogenous leukemia in
chronic phase: Increased risk for relapse associated with T-cell deple-
15. Lindahl-Kiessling K, Saffenberg J: Inability of UV-B irradiated
lymphocytes to stimulate allogeneic cells in mixed lymphocyte culture.
Int Arch Allergy Appl Immunol 41:670, 1971
16. Kahn RA, Duffy BF, Rodey GG: Ultraviolet irradiation of
platelet concentrates abrogates lymphocyte activation without affecting
platelet function in vitro. Transfusion 25: 547, 1985
17. Levin D, Gershon H: Interleukin production by neonatal spleen
cells during and as a result of antigen presentation. The effect of
18. Deeg HJ, Sigarouindua M: Ultraviolet B-induced loss of HLA
class II antigen expression on lymphocytes in dose, time, and locus
Elmets CA: Cell membrane is a major locus for ultraviolet B-induced
20. Deeg HJ, Bazar L, Sigarouindua M, Cohn M, Cotter-Fox M: Ultraviolet B light inactivates bone marrow T
lymphocytes but spare hematopoietic precursor cells. Blood 73:369, 1989
21. Fujihara M, Takahashi TA, Azuma M, Ogiso C, Mackaw TL,
Yogita H, Okumura K, Sekiguchi S: Decreased inducible expression of
CD80 and CD86 in human monocytes after ultraviolet-B irradiation: Its
22. Kao KJ: Effect of leukocyte depletion and UV-B irradiation on
alloantigenicity of major histocompatibility complex antigens in plate-
23. Deeg HJ, Aprite J, Graham TC, Appelbaum FR, Storb R: Ultraviolet irradiation of blood prevents transfusion-induced sensitiza-
alloimmunization in dogs with systemic cyclosporine and by UV-
allograft by treatment of recipients with ultraviolet irradiated donor
26. Kao KJ: Induction of humoral immune tolerance to major
histocompatibility complex antigens by transfusions of UVB-irradiated
27. Kao KJ, Scornick JC: Accurate quantitation of the low number of
white cells in white cell-depleted blood components. Transfusion
29:774, 1989
28. Zucali JR, Moreb J, Gibbons W, Alderman J, Suresh A, Zhang Y,
Shelby B: Radioprotection of hematopoietic stem cells by interleukin-1.
29. Kao KJ: Characterization of immune tolerance induced by transfu-
30. Sprent J, Schaefer M, Gao EK, Korgold R: Role of T cell subset
in lethal graft-versus-host disease directed to class I versus class II H-2
31. Sprent J, Schaefer M, Lo D, Korgold R: Properties of purified T
cell subsets: II. In vivo responses to class I vs. class II H-2 differences. J
32. Sprent J, Hurd M, Schaefer M, Heath W: Split tolerance in spleen
33. Sprent J, Korgold R: Murine models for graft-versus-host
disease, in SJ Forman, KG Blume, ED Thomas (eds): Bone Marrow
Transplantation, Boston, MA, Blackwell, 1994, p 220
34. Atkinson K: Reconstruction of the haemopoietic and immune
systems after marrow transplantation. Bone Marrow Transplant 5:209, 1990
35. Lemischka IR, Raulet DH, Mulligan RC: Developmental poten-
tial and dynamic behavior of hematopoietic stem cells. Cell 45:917, 1986
36. Salgame P, Abrams JS, Claytoner G, Goldstein H, Convit J,
Modlin RL, Bloom BR: Differing lymphokine profiles of functional
37. Moshmann TR, Cherwinski H, Bond MW, Giedlin MA, Coffman
RL: Two types of murine helper T cell clones. I. Definition according to
profiles of lymphokine activities and secreted proteins. J Immunol
136:2348, 1986
38. Romagnani S: Induction of Th 1 and Th 2 responses: a key role
39. Maizels RM, Bundy DA, Selkirk ME, Smith DF, Anderson RM:
Immunological modulation and evasion by helminth parasites in human
Current Opin Immunol 7:793, 1995
16:374, 1995
42. Moshman TR, Sad S: The expanding universe of T-cell subsets.
Th1, Th2 and more. Immunol Today 17:138, 1996
43. Chen N, Field HH: Enhance type 2 and diminished type 1
cytokines in neonatal tolerance. Transplantation 59:933, 1995
44. Kusaka S, Grailer AP, Fechner Jr, JH, Burlington WJ: Evidence
for a possible Th2 bias in human renal transplant tolerance. Transplant
Proc 27:225, 1995
45. Donckier VD, Wissing M, Bruyns C, Abramowicz D, Lybin M,
Vanderhaeghen ML, Goldman M: Critical role of interleukin 4 in the
induction of neonatal transplantation tolerance. Transplantation 59:
1171, 1995
46. Dallman MJ: Cytokines and transplantation: Th1/Th2 regulation
of the immune response to solid organ transplants in the adult. Curr
Opin Immunol 7:632, 1995
47. Pan L, Delmonte J, Jalonen CK, Ferrara JL: Pretreatment of
donor mice with granulocyte colony-stimulating factor polarizes donor
T lymphocytes toward type-2 cytokine production and reduces the
S, Higa T, Sakudara K, Asaka M: The important balance between
cytokines derived from type 1 and type 2 helper T cells in the control of
49. Blazar BR, Korngold R, Vallera DA: Recent advances in graft-versus-
50. Krenger W, Hill GR, Ferrara JLM: Cytokine cascades in acute
graft-versus-host disease. Transplantation 64:553, 1997
51. TRAP Study Group: Leukocyte reduction and ultraviolet B