Interleukin-2 Inhibits Graft-Versus-Host Disease–Promoting Activity of CD4+ Cells While Preserving CD4- and CD8-Mediated Graft-Versus-Leukemia Effects

By Megan Sykes, Mark W. Harty, Gregory L. Szot, and Denise A. Pearson

We have recently shown that a short course of high-dose interleukin-2 (IL-2) can markedly inhibit the graft-versus-host disease (GVHD)-promoting activity of donor CD4+ T cells. The difficulty in dissociating GVHD-promoting from graft-versus-leukemia (GVL) effects of allogeneic donor T cells currently prevents clinical bone marrow transplantation (BMT) from fulfilling its full potential. To test the capacity of IL-2 treatment to promote such a dissociation, we have developed a new murine transplantable acute myelogenous leukemia model using a class I major histocompatibility complex-positive BALB/c Moloney murine leukemia virus-induced promonocytic leukemia, 2B-4-2. BALB/c mice receiving 2.5 x 10^6 2B-4-2 cells intravenously 1 week before irradiation and syngeneic BMT died from leukemia within 2 to 4 weeks after BMT. Administration of syngeneic spleen cells and/or a 2.5-day course of IL-2 treatment alone did not inhibit leukemic mortality. In contrast, administration of non-T-cell–depleted fully allogeneic B10 (H-2b) spleen cells and T-cell–depleted B10 marrow led to a significant delay in leukemic mortality in IL-2–treated mice. In these animals, GVHD was inhibited by IL-2 treatment. GVL effects were mediated entirely by donor CD4+ and CD8+ T cells. Remarkably, IL-2 administration did not diminish the magnitude of the GVL effect of either T-cell subset. This was surprising, because CD4-mediated GVHD was inhibited in the same animals in which CD4-mediated GVL effects were not reduced by IL-2 treatment. These results suggest a novel mechanism by which GVHD and GVL effects of a single unprimed allogeneic T-cell subset can be dissociated; different CD4 activities promote GVHD and GVL effects, and the former, but not the latter activities are inhibited by treatment with IL-2.

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against this tumor are fully preserved in IL-2-treated mice in which CD4-dependent GVHD is inhibited by treatment with IL-2.

MATERIALS AND METHODS

Animals. Female C57BL/10 (B10, H-2d, K1P'D') and BALB/c (H-2b, K1P'D') mice were purchased from the Frederick Cancer Research Facility, Frederick, MD. Animals were housed in sterilized microisolator cages as described.8

BMT. BMT was performed as previously described with modifications described below. Recipient mice, aged 9 to 12 weeks, were irradiated (8-Gy whole body irradiation [WBI], 107Cs source, 1.1 Gy/min) and reconstituted with BMC and spleen cells prepared as described.9 Host-type (BALBk) BMC and allogeneic B10 BMC were TCD using rat antimouse CD4 IgG2b monoclonal antibody (MoAb) GK1.514 plus rat antimouse CD8 (Ly2.2-specific IgG2a MoAb 2.431 and rabbit C, as previously described.9 One milliliter of diluted antibody and C was used for 5 × 107 BMC. Because previous studies showed that TCD host-type BMC enhanced the protective effect of IL-2 without impairing allograftment, we co-administered 5 × 107 TCD host-type BMC in all experiments in which allogeneic CD4+ cells were given. For depletion of CD4+ and/or CD8+ spleen cells, 1 mL of diluted antibody and C was used for 107 splenocytes. The inocula used in each experiment are described in the Results section and figure legends. Animals in each group were randomized into various cages as previously described,9 and survival was monitored daily for 100 days.

2B-4-2 leukemia. 2B-4-2 is a tumor cell line derived in a pristane-primed BALB/c mouse that received an intravenous (IV) injection of Maloney Murine leukemia virus (M-MuLV).14 The cell line was kindly provided to us by Dr Linda Wolff (National Cancer Institute, Bethesda, MD). The cells were injected intraperitoneally to BALB/c mice, and ascites was produced. This ascites was pooled and frozen, and a fresh vial was thawed for use in each experiment. The thawed 2B-4-2 cells were expanded in culture for 1 to 2 weeks in RPMI supplemented with 10% fetal calf serum, 100 U/mL penicillin, 100 μg/mL streptomycin, 2 mM L-glutamine, 1 mM L-sodium pyruvate, 0.09 mM L-nonessential amino acids, and 0.01 mM L-HEPES buffer. 2B-4-2 cells were administered intravenously 1 week before irradiation and BMT.

In some experiments, carcasses of animals that succumbed were saved in 10% formalin. After completion of the survival study, histologic examination was performed by an individual who was unaware of the experimental group to which the animals belonged. Gross abnormalities were recorded, and the only abnormality that was described was a grossly observable tumor in the spleen. This tumor is fully preserved in IL-2-treated mice.

Statistical analysis. Survival data were analyzed using the Kaplan-Meier method of life table analysis, and statistical analysis was performed with the Mantel-Haenzsen test.

RESULTS

Characteristics of transplanted 2B-4-2 leukemia. 2B-4-2 is a tumor cell line derived from a pristane-primed BALB/c mouse that received an IV injection of M-MuLV.16 Previous characterization of these tumors led to their categorization as promonocytic cells based on morphology, cell surface markers, and enzyme-staining characteristics.16 Similar to previously reported results,16 our own FCM analyses showed these cells to be Thyl-negative, surface IgM-negative, Mac1-positive, class I MHC-positive, and class II MHC-positive. They did not express the granulocyte marker Gr-1 (data not shown). After thawing and expanding 2B-4-2 cells in vitro for 1 week, several cell numbers were transferred intravenously to BALB/c recipients. As shown in Table 1, the speed of mortality was a function of cell dose, and 5,000 2B-4-2 cells were sufficient to kill all recipients. One recipient of 5 × 107 cells was sacrificed on day 14, and its marrow,
performed at 8, 14, and, when available, 21 days post-BMT.

Thus, our model resembled advanced acute leukemia, in which irradiation followed by autologous BMT led to a small, but statistically significant, prolongation of survival. Similar results were obtained in a repeat experiment. Autopsies showed marked splenic enlargement in most animals (14/15) in both groups, with no apparent effect of IL-2 treatment.

Lack of antileukemic effect of IL-2 given with syngeneic marrow. We have recently shown that a short course of high-dose IL-2 can markedly inhibit GVHD in a variety of strain combinations involving a full MHC mismatch, including B10 → BALB/c.13 To determine whether such a course of IL-2 would, on its own, mediate an antileukemic effect against 2B-4-2, we compared leukemic mortality in control and IL-2-treated BALB/c mice that had received 7.5 × 105 2B-4-2 cells 1 week before lethal WBI and rescue with TCD syngeneic BMC. As shown in Fig 2, a 2.5-day course of human recombinant IL-2, 50,000 U per dose, had no effect on leukemic mortality when given with TCD syngeneic BMC alone. Similar results were obtained in a repeat experiment. Autopsies showed marked splenic enlargement in most animals (14/15) in both groups, with no apparent effect of IL-2 treatment.

GVL effect of allogeneic lymphocytes. To determine whether or not allogeneic lymphocytes could mediate a GVL effect, we also evaluated the effect of administering fully MHC (plus multiple minor)-mismatched B10 BM and spleen cells in the experiment shown in Fig 2. We have previously shown that IL-2 can inhibit GVHD mortality in this B10 → BALB/c strain combination,13 so IL-2 was given to recipients of these allogeneic inocula to minimize GVHD. As shown in Fig 2, control animals receiving a mixture of TCD syngeneic (BALB/c) BMC, B10 BMC, and B10 spleen cells without WBC count (54.7 × 106/mL) associated with a slightly reduced platelet count. Only one 2B-4-2 recipient was analyzed on day 21, and it also showed a markedly elevated WBC count (42.9 × 106/mL) and a slightly reduced platelet count compared with simultaneous BMT controls. At autopsy, animals showed varying degrees of splenic enlargement.

Table 1. Leukemic Mortality in Untreated and Irradiated/BMT BALB/c Recipients of Various 2B-4-2 Doses

<table>
<thead>
<tr>
<th>Group Administered*</th>
<th>Untreated</th>
<th>Survival (d)</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of 2B-4-2 Cells</td>
<td></td>
<td>WBI/BMT1</td>
</tr>
<tr>
<td>1</td>
<td>0</td>
<td>&gt;140, &gt;140, &gt;140 &gt;107, &gt;107, &gt;107, &gt;107</td>
</tr>
<tr>
<td>2</td>
<td>5,000</td>
<td>28, 33, 115</td>
</tr>
<tr>
<td>3</td>
<td>5 × 10⁴</td>
<td>20, 24, 26</td>
</tr>
<tr>
<td>4</td>
<td>5 × 10⁵</td>
<td>17, 19, 19</td>
</tr>
<tr>
<td>5</td>
<td>5 × 10⁶</td>
<td>10, 17</td>
</tr>
<tr>
<td>6</td>
<td>5 × 10⁷</td>
<td>8, 13</td>
</tr>
</tbody>
</table>

Abbreviation: ND, not determined.

* 2B-4-2 cells were administered intravenously on day 0.
† Number of days animals survived after 2B-4-2 administration is shown. On day 7, animals received 8-Gy WBI, followed by IV administration of 5 × 10⁵ BALB/c BMC. These animals were evaluated in a separate experiment from that in which the Untreated group was tested.
tumor showed 100% survival. Animals receiving similar in-
oula, but that had received 2B-4-2 cells one week before
irradiation and BMT, showed 100% mortality from
leukemia by day 32 (Fig 2). However, this represented a
significant delay in leukemic mortality compared with
control IL-2–treated recipients of syngeneic BMC alone,
which all died by day 17 (P < .005), indicating that
allogeneic cells mediated a GVL effect in IL-2–treated
mice. Similar results were obtained in a repeat experi-
ment. In contrast with autopsies performed on control
leukemic mice, those performed on 18 carcasses from
leukemic recipients of allogeneic inocula compared with
control IL-2-treated mice. Similar numbers of untreated
or CD8+ T cells mediated GVL effects in IL-2–treated
mice. We next wished to determine which cell
mediated a GVL effect in IL-2–treated mice. The magnitude
of the GVL effect of either CD4+ or CD8+ T cells
mice, so that survival was indistinguishable from that of
animals receiving BMC alone (P = .75; P < .01 comparing
recipients of CD4- and CD8-depleted spleen cells with
recipient of splenocytes depleted of either CD4+ or CD8+ cells).
Fluorescence-activated cell sorter (FACS) analyses showed
that survival was indistinguishable from that of
animals receiving BMC alone (P = .75; P < .01 comparing
recipients of CDC-depleted or CD8-depleted B10 spleen cells
with recipients of no B10 spleen cells). Depletion of both T-cell subsets, on the other hand,
led to complete abrogation of the GVL effect of B10 spleen
cells, so that survival was indistinguishable from that of
animals receiving BMC alone (P = .75; P < .01 comparing
recipients of CD4- and CD8-depleted spleen cells with
recipients of splenocytes depleted of either CD4+ or CD8+ cells).
Fluorescence-activated cell sorter (FACS) analyses showed
that depletion of each subset was 94% to 99.5% complete.
In addition, the complete loss of GVL effects in recipients
of B10 spleen cell inocula depleted of both subsets indicates
that failure to see complete abrogation of the GVL effect
with depletion of any one subset was not simply due to
incomplete depletion. Thus, both CD4+ and CD8+ T cells
mediated a GVL effect in IL-2–treated mice.

Necropsies performed on 6 of 8 leukemic animals receiv-
ing only BMC showed marked splenic enlargement; similar
enlargement was seen in 8 of 11 leukemic mice receiving
BMC with B10 spleen cells depleted of CD4+ and CD8+
cells. In contrast, 0 of 11 animals receiving C-treated
B10 spleen cells showed splenic enlargement at death; 0 of
12 recipients of CD4-depleted and 5 of 13 recipients of CD8-
depleted B10 spleen cells showed marked splenic
enlargement.

As shown in Fig 3B, IL-2 inhibited GVHD mortality in
nonleukemic controls receiving both CD4+ and CD8+ T cells
in the same experiment. Because all of the animals in the
top panel of Fig 3 were IL-2–treated, this experiment shows
that IL-2 can inhibit GVHD while preserving GVL effects
of both CD4+ and CD8+ T cells.

The magnitude of CD4-mediated and CD8-mediated GVL
effects is not reduced by IL-2 treatment. Our next studies
were performed to determine whether or not IL-2 inhibited
the magnitude of the GVL effect of either CD4+ or CD8+
Fig 3. (A) BALB/c mice received $2.5 \times 10^5$ 2B-4.2 cells intravenously on day -7, followed by 8-Gy WBI on day 0, and IV administration of $7 \times 10^6$ TCD B10 BMC and $5 \times 10^6$ TCD BALB/c BMC alone (--, n = 5) or with $12 \times 10^6$ B10 spleen cells treated with C only (-----, n = 8; inoculum contained 2.19 x $10^6$ CD4$^+$ and 0.89 x $10^6$ CD8$^+$ cells); anti-CD4/C (-----, n = 8; inoculum contained 0.007 x $10^6$ CD4$^+$ and 0.85 x $10^6$ CD8$^+$ cells); anti-CD8/C (-----, n = 8; inoculum contained 2.0 x $10^6$ CD4$^+$ plus 0.048 x $10^6$ CD8$^+$ cells), or anti-CD4 plus anti-CD8/C (-----, n = 8; inoculum contained a total of 0.043 x $10^6$ CD4$^+$ and CD8$^+$ cells). All mice received IL-2, $5 \times 10^4$ Cetus Units intraperitoneally, twice daily from day 0 to 2 (5 doses). (B) IL-2 protects against GVHD in nonleukemic control mice in the same experiment. Animals received similar inocula as those in the top panel, without receiving tumor. Recipients of BMC only (-----; n = 4); BMC plus C$^+$-treated B10 spleen cells (- - - - -; n = 4); BMC plus C$^+$-treated B10 spleen cells plus IL-2 (-----; n = 8).

We next evaluated the possible effect of IL-2 on the magnitude of the GVL effect of B10 CD4$^+$ cells in this model. This experiment was more difficult to perform, as CD4$^+$ cells can cause GVHD in the absence of CD8$^+$ cells in this strain combination, making it difficult to distinguish GVHD mortality from GVHD mortality in animals not receiving IL-2 treatment. However, by comparison with nonleukemic controls, we were able to show that the magnitude of the GVL effect (which was small in the two experiments performed that are combined in Fig 5) of B10 CD4$^+$ cells was not reduced by treatment with IL-2. The GVL effect achieved statistical significance in IL-2-treated mice ($P < .05$ comparing IL-2-treated leukemic recipients of CD8-depleted B10 spleen cells to leukemic recipients of BMC only). However, statistically significant survival prolongation was not observed in the non-IL-2-treated recipients of CD8-depleted B10 spleen cells ($P = .15$), most likely because several early GVHD-related mortalities were observed in this group. We believe these early (<10 days) mortalities were GVHD-induced, because similar early mortality was observed for the nonleukemic GVHD controls receiving similar treatment (Fig 5B). In contrast, no early mortalities were observed in IL-2-treated nonleukemic control recipients of B10 CD4$^+$ cells (Fig 5B) or in IL-2-treated leukemic recipients of these cells (Fig 5A), allowing detection of a statistically significant GVL effect.

**Chimerism in long-term survivors.** Peripheral WBC chimerism was evaluated in long-term surviving animals in three separate experiments. One control and one IL-2-treated nonleukemic recipient of B10 spleen cells plus TCD syngeneic and allogeneic BMC in which peripheral WBC were phenotyped 122 days post-BMT showed complete (>99%) donor-type hematopoietic reconstitution. Among animals typed at 177 and 293 days post-BMT, leukemic (n = 1) and nonleukemic (n = 7) recipients of CD4-depleted B10 spleen cells and TCD-B10 BMC with or without TCD-BALB/c marrow and IL-2 all showed complete (>99%) donor repopulation of the monocyte, neutrophil, and lymphocyte lineages. Donor cells repopulated 91% of lymphocytes...
and granulocytes and 99% of monocytes of an animal that received TCD-B10 BMC alone without spleen cells. Three of five control recipients of TCD B10 plus TCD-BALB/c marrow without B10 spleen cells showed mixed chimerism in all lineages, whereas the remaining two mice repopulated only with host-type WBC.

**DISCUSSION**

In this study, we have developed a new transplantable acute myelogenous leukemia model to show that the GVL effect of both CD4+ and CD8+ T cells is completely preserved in BMT recipients treated with a short course of high-dose IL-2 that inhibits CD4-dependent GVHD. This is, to our knowledge, the first in vivo demonstration that the GVL effect and GVHD-producing capacity of unprimed bulk T-cell populations consisting of a single subset (ie, CD4+) can be dissociated. This result suggests a novel mechanism by which GVHD might be avoided while GVL effects of alloreactive donor T cells could be exploited for their GVL effects in HLA-mismatched clinical BMT.

Because a virally-induced tumor was used for our studies, reactivity directed against tumor-associated antigens could have been present. However, host-type T cells administered in BALB/c spleen cell inocula did not mediate a detectable antileukemic effect in IL-2–treated BMT mice. Host-restricted T cells educated in a host thymus were administered in these syngeneic spleen cell inocula, and should have been able to optimally recognize virus-specific peptides presented on the host-type 2B-4-2 leukemia cells. In contrast, donor T cells in allogeneic spleen cell inocula are educated in a donor thymus, and therefore, recognize peptide antigen in the context of donor MHC. Therefore we believe that viral peptide-specific reactivity on its own cannot explain the GVL effect of allogeneic CD4+ or CD8+ T cells, and that the majority of this GVL effect is caused by alloreactivity. However, we cannot rule out the possibility that interactions between virus-specific and alloreactive T cells could explain the greater GVL effect of allogeneic T cells. In addition, it is possible that the use of a less rapidly lethal leukemia model would permit less potent responses directed against virus-specific antigens to mediate a detectable GVL effect. However, our use of a rapidly lethal leukemia established in recipients 1 week before irradiation and BMT permits detection of only the most potent antileukemic activity, such as that caused by recognition of MHC alloantigens. In another leukemia model, we observed antileukemic effects only in the presence of MHC mismatches, and not when MHC-matched allogeneic BMC were given. T cells that react against any particular MHC/peptide complex, such as those formed by tumor-associated antigens or minor histocompatibility antigens plus self MHC, are quite rare. In contrast, the frequency of unprimed T cells reacting against allogeneic MHC antigens is several orders of magnitude higher than that directed against even multiple minor histocompatibility antigens plus “self” MHC. Therefore, more potent antileukemic effects would be expected in MHC-mismatched BMC, as we have observed. Whereas antiminnor alloreactivity clearly mediates antileukemic effects in human BMT, it is likely that more potent GVL effects would be achieved if BMT could be performed safely across wider HLA disparities. Indeed, lower leukemic relapse rates have been observed for related or unrelated single HLA antigen-mismatched BMC recipients than for HLA-identical transplant recipients. Unfortunately, these improved GVL effects are mitigated by increased GVHD, resulting in no survival advantage compared with HLA-matched transplants. The results reported here suggest a method of attenuating the GVHD-producing activity of allogeneic CD4+ T cells while preserving the GVL effects of the same T-cell subset in HLA-mismatched transplantation. With a few exceptions, CD4+ cells have been found to play a critical role in inducing acute GVHD across complete MHC barriers in
Fig 5. IL-2 does not influence the GVL effect of B10 CD4⁺ T cells in BALB/c mice. BALB/c mice received 2.5 x 10⁶ 2B-4-2 cells intravenously on day -7, followed by 8-Gy WBI on day 0, and IV administration of 10 x 10⁶ TCD-B10 BMC, 5 x 10⁶ TCD-BALB/c BMC, with or without 15 x 10⁶ CD8-depleted B10 spleen cells on day 0. Spleen cell inocula contained 0.4% residual CD8⁺ cells and 12.0% CD4⁺ cells. IL-2 was administered to the indicated groups at a dose of 5 x 10⁶ Cetus Units intraperitoneally, twice daily from day 0 to 2.5 (5 doses). (A) Treatment groups: --; n = 13, leukemia and BMC only; ---; n = 10, leukemia, BMC, and CD8-depleted B10 spleen cells; - - - ; n = 10, leukemia, BMC, CD8-depleted B10 spleen cells, and IL-2 treatment. (B) Treatment groups: --; n = 10, BMC only, no leukemia; ---; n = 15, BMC, CD8-depleted spleen cells, no leukemia; - - - ; n = 15, BMC, CD8-depleted B10 spleen cells, IL-2, no leukemia.

We have shown CD4 dependence for acute GVHD in the B10 → BALB/c strain combination used here. Similar CD4 dependence of full haplotype-mismatched GVHD has been observed in preliminary studies in miniature swine (T. Sablinski and D.H. Sachs, unpublished data, September 1992). Thus, in most cases, inhibition of CD4 functions with IL-2 could reduce GVHD without diminishing CD8-mediated GVL effects, as we have previously shown for the EL4 leukemia/lymphoma in the A/J → B10 strain combination. We have shown that CD4-dependent GVHD can be inhibited by IL-2 in several fully MHC-mismatched strain combinations. In addition, when CD4⁺ cells were given without CD8⁺ cells in the A/J → B10 or in the B10 → BALB/c combination, IL-2 inhibited GVHD. However, before this information can be optimally utilized in HLA-mismatched clinical BMT, it will be necessary to gain a clearer understanding of the factors that determine CD4 dependence of GVHD, of which CD4 activities mediate GVHD (and GVL), and of which of these activities are inhibited by IL-2.

Therefore, an alternative approach to preserving GVL effects while inhibiting GVHD might involve selective CD4 depletion of allogeneic inocula. However, this latter approach has several potential disadvantages compared with IL-2 treatment: (1) preliminary studies in a miniature swine full haplotype-mismatched BMT model suggest that CD4 depletion eliminates GVHD, but also leads to a high incidence of failure of engraftment (T. Sablinski and D.H. Sachs, unpublished data), suggesting an important role for CD4⁺ cells in promoting alloengraftment in large animals. IL-2 treatment has been shown not to inhibit the engraftment-promoting effects of allogeneic T cells in mice. (2) CD4 depletion would remove CD4-mediated GVL effects. CD4⁺ T cells have been shown to participate in GVL reactions in
another model besides the one reported here. In contrast to results in pan-T-cell–depleted BMT, clinical GVHD can be reduced whereas GVL is apparently preserved when CD8+ T cells are depleted from HLA-matched sibling marrow, suggesting that CD4+ T cells may also mediate GVL effects in man. This is not surprising, because many human leukemias express class II MHC antigens. The susceptibility of the 2B-4-2 leukemia and the lack of susceptibility of EL4 to CD4-mediated GVL effects probably reflects the expression on 2B-4-2, but not on EL4, of class II MHC antigens (M. Sykes, D.A. Pearson, M.W. Harty, unpublished data, September 1991). Therefore, IL-2 treatment might provide a superior approach compared to selective CD4 depletion of allogenic marrow because of its potential to better preserve alloengraftment and GVL effects while avoiding GVHD in class II MHC-mismatched BMT.

In addition to the current result showing preservation of CD4-mediated GVL effects while CD4-mediated GVHD is inhibited in IL-2–treated mice, several other lines of evidence support the conclusion that not all CD4-mediated activities are inhibited by IL-2: (1) More complete inhibition of GVHD was observed in non-IL-2–treated recipients of CD4-depleted allogeneic spleen cells than in IL-2–treated recipients of undepleted spleen cells from the same allogeneic donors in this B10 → BALB/c strain combination. Indeed, we have observed that IL-2 does not inhibit CD4-mediated chronic GVHD in this combination. Thus, not all GVL-producing CD4 activity is inhibited by IL-2 treatment; (2) In an MHC-matched, multiple minor antigen disparate strain combination, B10 → C3H.SW, CD4+ T cells made a small contribution to GVHD, but IL-2 provided no GVHD protection, suggesting that the CD4-mediated component of GVHD in this strain combination was qualitatively distinct from the IL-2–sensitive CD4 functions that cause GVHD across MHC barriers; (3) In 1 of 10 fully MHC-mismatched strain combinations examined, B10 → A/J, IL-2 did not provide any protection against GVHD, which was CD4 dependent. Together, these results suggest that there are qualitatively different (not only quantitative) differences in the CD4 activities that mediate GVHD in the various strain combinations, and that some, but not all, of these activities, are inhibited by IL-2. Fortunately, the CD4 activities leading to GVL effects, at least in the 2B-4-2 model, are not susceptible to inhibition by IL-2.

What are the CD4 cell functions or subsets that are sensitive or resistant to the inhibitory effects of IL-2? CD4+ T cells can contribute to GVHD through both helper and cytolytic activities, and helper activities include several distinct patterns of cytokine production. Preliminary in vitro and in vivo studies suggest that IL-2 perturbs Th1 activity, may increase Th2 activity, and does not inhibit cytolytic activity of CD4+ T cells (M. Sykes, J. Szebeni, M. Wang, unpublished data, January 1994). In vivo studies showed that Th1 and not Th2 CD4+ clones can be prevented from responding to antigen if they are first exposed to IL-2. Therefore, we hypothesize that the GVL effect of B10 CD4+ cells against 2B-4-2 is caused by cytolytic activity, and that Th1 functions do not contribute to GVL, but play an essential role in producing GVHD. Indeed, the Th1-associated cytokines interferon-γ and IL-2 have been implicated as important mediators of GVHD pathophysiology. Several mechanisms have been proposed to explain this dissociation, including the recognition of tumor-associated or tissue-specific antigens. However, this study is the first, to our knowledge, to suggest that recognition of MHC alloantigens by unprimed bulk T-cell populations consisting of a single subset can be associated with separable GVHD-producing and GVL activities. A better understanding of this dichotomy might permit the performance of HLA-mismatched BMT to maximize donor availability and both CD4-mediated and CD8-mediated GVL effects while minimizing GVHD.

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**REFERENCES**


8. Sykes M, Rombick ML, Hoyles KA, Sachs DH: In vivo adminis-
24. Tomita Y, Sachs DH, Sykes M: Myelosuppressive conditioning is required to achieve engraftment of pluripotent stem cells contained in moderate doses of syngeneic bone marrow. Blood (in press)
35. Thiele DL, Bryde SE, Lipsky PE: Lethal graft-vs-host disease induced by a class II MHC antigen only disparity is not mediated by cytotoxic T cells. J Immunol 141:3377, 1988
44. Höller E, Kolb HJ, Hintermeier-Knabe R, Mittermueller J,


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