Prevention of Murine Graft-Versus-Host Disease and Bone Marrow Alloengraftment Across the Major Histocompatibility Barrier After Donor Graft Preincubation With Anti-LFA1 Immunotoxin

By Bruce R. Blazar, Stephen F. Carroll, and Daniel A. Vallera

We have investigated the effects of the in vitro depletion of LFA1 positive cytolytic T lymphocytes, natural killer (NK) cells, and monocytes on the afferent phase of graft-versus-host disease (GVHD). Lethal GVHD was induced across the murine major histocompatibility complex by injecting C57BL/6 (H-2b) bone marrow (BM) cells (a source of stem cells) and splenocytes (S) (a source of T cells) into lethally irradiated B10.BR (H-2r) recipients. Because anti-LFA1 does not bind complement (C) effectively, we conjugated anti-LFA1 α chain monoclonal antibody (MoAb) to ricin toxin A chain (RTA) as a means of facilitating target cell elimination. A 2-hour preincubation of C57BL/6 bone marrow/spleen (BMS) with anti-LFA1-RTA in the presence of ammonium chloride (a potentiator of immunotoxin toxicity), but not a control immunotoxin (IT), reduced CTL activity by greater than 2 logs, significantly reduced NK cell activity, and prevented B10.BR mice from developing GVHD. Depletion of target cells by toxin-labeled-MoAb and not the blockade of the LFA1 molecule by the anti-LFA1 MoAb accounted for our results, because incubating cells with IT in the absence of a potentiatior had no effect on GVHD prevention. In contrast, C57BL/6 recipients of C3H BMS grafts only partially benefitted from anti-LFA1-RTA preincubation, demonstrating that in this system, different cells not expressing LFA1 were involved in GVHD generation. The same findings observed with anti-LFA1-RTA preincubation were observed with preincubation with L-leucyl-L-leucine methyl ester, a chemical compound eliminating cytolytic cells, providing further support that GVHD induction in the C3H/HeJ into C57BL/6 system is not entirely mediated by classical cytolytic T cells. We next tested anti-LFA1-RTA in a model devised to measure its effect on alloengraftment (B10.BR recipients given lower doses of irradiation). Anti-LFA1-RTA BM preincubation selectively reduced alloengraftment in the model. This observation, combined with experiments showing that LFA1-RTA preincubation, but not anti-Thy1.2 + C or control IT preincubation, reduced colony-forming unit-spleen formation, indicates that anti-LFA1 α chain IT may remove accessory cells or stem cells critical to engraftment. Still, anti-LFA1-RTA may be useful for clinical GVHD prevention when combined with positive selection techniques designed to enrich for stem cells.

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In vitro PAN T-cell depletion (TCD) of donor marrow is an effective means of reducing or preventing murine3 and human graft-versus-host disease (GVHD). However, TCD is associated with an increase in engraftment problems.4 Therefore, in previous studies, we tested a chemical method for depletion of cytolytic cell populations using a lysosomotropic compound, L-leucyl-L-leucine methyl ester (LLME), described by Thiele and Lipsky.5,6 This compound is highly efficient in eliminating cytolytic T cells (CTL), natural killer (NK) cells, and monocytes.4,5 In vitro incubation of donor bone marrow (BM)/spleen (S) inoculum with LLME has been shown to prevent GVHD in several donor-recipient strain combinations in which class I and/or minor but not class II only histocompatibility antigen recognition by CTL is involved.1-13 Moreover, donor graft incubation with LLME before BM transplantation (BMT) has not interfered with alloengraftment in murine recipients of semi-allogeneic,14 minor histocompatibility antigen disparate,15 or in the context of major histocompatibility complex (MHC) class I + II + multiple minor antigen donor disparities.4 However, in a canine autologous BMT model, at doses necessary to eliminate cytolytic cell function, LLME substantially reduced the number of progenitor cell colonies even when accessory cells were added back to the culture.15 Recent data in humans have supported the observed inhibition of progenitor cell colony formation in the canine model.16 In addition, a minority of patients had an apparent partial CTL resistance to LLME treatment.16 Therefore, we investigated an alternative approach to eliminating CTL, NK cells, and monocytes by antibody-directed targeting of the LFA1 α chain (CD11a) molecule.5,16 Because the murine LFA1 monoclonal antibody (MoAb) used for these studies does not bind complement (C),8 we constructed an anti-LFA1 immunotoxin (IT) as a means of killing the target cells. ITs are MoAb covalently linked to catalytic toxins. We used ricin toxin A chain (RTA) that only requires a single molecule in the cytosol for cell death.21 RTA is not reactive with receptors on human or mouse cells, and thus does not efficiently penetrate the cell membrane unless attached to an MoAb that recognizes a cell surface antigen which is capable of internalization.22,23 We determined the effect of anti-LFA1-RTA preincuba-


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tion of donor grafts on GVHD prevention and allograftme

MATERIALS AND METHODS

Preparation of anti–T-cell ITs. Anti-LFA1 α chain MoAb (FD441.8) (Dr Frank Fitch, University of Chicago, Chicago, IL)20 and anti-Thy1.2-RTA (obtained from the American Type Culture Collection [ATCC], Rockville, MD) are rat IgG2b MoAb. Anti-Thy1.2-RTA and anti-LFA1-RTA were synthesized by linking purified MoAb to RTA or RTAα (a purified fraction of RTA with reduced carbohydrate content). Both had similar in vitro and in vivo activities against mouse cells. The conjugation technique has been previously described in detail.24-26 H65 is a control mouse IgG2a antihuman CD5 MoAb27 that was conjugated to RTA. Mel-RTA is a control IT made by linking mel, a mouse IgG2a MoAb recognizing human melanoma antigen,22 to RTA. These reagents are referred to as control IT. We have noted no differences in rat, mouse, or isotype control MoAb used as control IT.

Gel analysis. To assess the purity of IT, analysis was performed on a 10% polyacrylamide gel electrophoresis (PAGE) plate (Daiichi Pure Chemicals, Tokyo, Japan) using a Mini Protean II slab cell (BioRad, Richmond, CA) as previously described.27 Quantitation of the bands was performed by densitometric analysis using a Gel Scan Soft Pack Module Software Package (Beckman Instruments, Fullerton, CA).

Indirect immunofluorescence. Because IT does not immediately destroy target cells, the binding of anti-Thy 1.2-RTA or anti-LFA1-RTA was determined by standard indirect immunofluorescence techniques. For in vitro analysis, 105 Thy 1.2+ and LFA1+ EL4 cells were incubated with 10 μg/mL IT or a previously determined saturating amount of MoAb for 30 minutes at 4°C. The cells were then washed and incubated for 30 minutes at 4°C with 50 μL of a 1/50 dilution of fluoresceinisothiocyanate (FITC)-conjugated goat anti-rat IgG (GARG). Cells were washed three times, diluted in buffer, and analyzed. Flow cytometry was performed on the FACSscan (Becton Dickinson, Mountain View, CA) as previously described.2 Background binding using a labeled control antihuman T-cell MoAb, 3AIE,28 was subtracted from the results.

Mice. C57BL/6 (H-2b) and C3H/HeJ (H-2k) donor mice, and B10.BR/SgSnJ (H-2d) recipient mice were purchased from Jackson Laboratories. Engraftment was measured by analyzing the cell number culture and the logarithm of the percentage of nonresponding (negative) cultures. When plotted according to the zero-order kinetics, the data define a straight line with a slope equal to the frequency of CTL precursors.

NK cell activity was measured as previously described.2 Re- sponding cells were plated with Na16CrO4-labeled YAC-1 target cells. Four hours after adding targets, 100 μL of supernatant was removed and counted in a gamma counter. The percent specific cytotoxicity was calculated.

Chimerism analysis post-BMT. Chimerism of peripheral blood mononuclear cells was analyzed by examining the cell surface binding of anti–H-2 MoAb linked to fluorochromes on a FACScan. Anti–H-229 (clone 11-4.1, mouse IgG2a; ATCC) or anti–H-229 (clone EHL14, mouse IgG2b; provided by Dr T.V. Rajan, Albert Einstein University, New York, NY) was directly conjugated to FITC or phycoerythrin (PE), respectively, as previously described.2 3AIE was conjugated to FITC or PE to determine background binding.

Hematologic evaluation of recipients post-BMT. Fifty microliters of peripheral blood was obtained by retro-orbital venipuncture on days 7, 14, and 28 post-BMT. Leukocyte number and morphology were determined by examination of Wright-Giemsa stained slides.2 Hematocrit values were determined by capillary tube red blood cell to plasma volume ratios after centrifugation.

Skin grafting. To measure immunocompetence, donor and
third party skin grafting was performed. Rejection of donor and third-party (BALB/cJ-H-2^b) skin was tested (n = 3 mice/group) as previously described.22

Statistical analysis. Groupwise comparisons of continuous data were made using the Student's t-test. The computer program for compiling life table and statistical analysis by the log-rank test was provided by Dr Bruce Bostrom (Department of Pediatrics, University of Minnesota, Minneapolis).

RESULTS

Gel analysis. Sodium dodecyl sulfate (SDS) PAGE under nonreduced conditions was performed. Gel profiles from scanning anti-LFA1-RTA showed three bands of IT exceeding 150 Kd, the molecular weight of anti-LFA1 MoAb alone (not shown). The IT bands from the smallest to the largest represented 31%, 27%, and 13% of the total protein applied to the gradient. Nineteen percent free antibody and less than 5% free RTA were present. Gel profiles for this lot of anti-Thyl.2-RTA have been reported.2

Binding of IT to the EL4 cell line as measured by indirect immunofluorescence. Eighty-nine percent of the LFA1 positive EL4 cell line bound anti-LFA1-RTA with a similar binding intensity (mean fluorescent channel) to anti-LFA1 MoAb, which bound 90% of EL4 cells (not shown). These data suggest that the conjugation procedure did not substantially alter MoAb binding.

In vitro studies with IT. Anti-Thyl.2-RTA potency and specificity was measured by PHA mitogenesis using splenocytes from a Thyl.2 ^c strain (C57BL/6) and a Thyl.2 ^a strain (AKR) as previously reported.2 In brief, Thyl.2-RTA completely eliminated PHA responsiveness in C57BL/6 splenocytes at a concentration of 10 ^g/mL, while reducing AKR PHA responsiveness by 19.8%. As shown in Table 1, anti-LFA1 was highly effective in reducing the CTL precursor frequency by greater than 2 logs and eliminating NK activity. In contrast, a control IT had negligible effects. In addition, anti-LFA1-RTA pretreatment inhibited NK function, as did anti-NK1.1-RTA in previous studies.30

The efficacy of anti-LFA1-RTA incubation of donor grafts with IT in preventing GVHD induced in B10.BR recipients of C57BL/6 allografts. GVHD was induced by the injection of C57BL/6 BM and spleen cells or LN T cells (Fig 1). As few as 5 x 10^6 splenocytes or 10^6 lymph node T cells resulted in the mortality of >70% of irradiated recipients, in contrast to the recipients of BM supplemented with 25 x 10^6 TCD splenocytes (n = 10/group). Because the mortality rate of mice given 15 to 25 x 10^6 nondepleted supplemental splenocytes was 100% before 5 weeks post-BMT, we chose the highest splenocyte cell dose tested to study the efficacy of anti-LFA1 pretreatment of donor grafts.

Cumulative actuarial survival data from two identical experiments in which bone marrow/spleen (BMS) grafts were preincubated with anti-LFA1-RTA (30 ^g/mL), anti-Thyl.2-RTA (10 ^g/mL), control IT, or control PBS are depicted in Fig 2. Overall, 90% of anti-LFA1-RTA treated BMS grafts survived the entire observation period. None of the mice had evidence of GVHD at any time period post-BMT. These results are similar to the 100% survival of anti-Thyl.2-RTA treated grafts. Recipients of control-RTA IT treated grafts had a median survival time of 40.5 days, with 20% of recipients surviving the observation period. Mice that received PBS-treated grafts had a median survival time of 33.9 days, with 11% surviving the observation period. There was no significant difference in survival in comparing these two different control groups, while each was significantly (P < 3 x 10^-7) lower than recipients of anti-LFA1-RTA or anti-Thyl.1.2-RTA. At all periods post-BMT, recipients of anti-LFA1-RTA or anti-Thyl.1.2-RTA treated grafts did not experience the progressive weight loss (especially prominent 12 to 13 days post-BMT) characteristic of murine GVHD (data not shown).

Long-term chimerism studies on recipients shown in Fig 2 were performed at least 6 weeks post-BMT (Table 2). They demonstrated that the IT treatment did not interfere with alloengraftment. Four of six mice in each group surviving the observation period were skin grafted. All mice rejected third party (BALB/c) skin grafts within 13 days of graft placement. Donor skin grafts were accepted in five of six mice that received anti-LFA1-RTA treated BMS grafts and in five of six mice that received anti-Thyl.1.2-RTA treated grafts. It is possible that the single mouse rejection in each group was due to a technical failure.

In a third experiment (not shown), recipients of anti-LFA1-RTA at a dose of 30 ^g/mL had a similar weight curve and similar survival curve as 10 ^g/mL recipients

Table 1. In Vitro Characterization of Anti-LFA1-RTA

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose (µg)</th>
<th>CTLp Frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nontreated</td>
<td>NA</td>
<td>1/16,362</td>
</tr>
<tr>
<td>LFA1-RTA</td>
<td>30</td>
<td>1/341,848</td>
</tr>
<tr>
<td>Control-RTA</td>
<td>30</td>
<td>1/18,885</td>
</tr>
<tr>
<td>Nondepleted</td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td>LFA1-RTA</td>
<td>1</td>
<td>13</td>
</tr>
<tr>
<td>LFA1-RTA</td>
<td>0.1</td>
<td>13</td>
</tr>
<tr>
<td>Control-IT</td>
<td>1</td>
<td>13</td>
</tr>
<tr>
<td>Control-IT</td>
<td>0.1</td>
<td>13</td>
</tr>
</tbody>
</table>

C57BL/6 S were incubated for 2 hours at 37°C with IT at the indicated concentrations. CTLp frequency and NK activity were determined as described in Materials and Methods.
We tested three doses of anti-LFA1-RTA for preincubation. In addition to the 10 or 30 μg/mL doses, we also studied 1 μg/mL, because this lower dose of anti-LFA1-RTA was sufficient to eliminate NK function (see Table 1). Survival data are depicted in Fig 5. In contrast to the highly efficacious effect of anti-LFA1-RTA in preventing GVHD induced by C57BL/6 splenocytes in irradiated B10.BR recipients, anti-LFA1-RTA incubation at concentrations of 10 or 30 μg/mL was only partially effective in preventing GVHD induced by C3H/HeJ grafts in irradiated C57BL/6 recipients. Recipients of anti-LFA1-RTA at the lowest concentration (1 μg/mL), control IT treated, or C' treated grafts died before 10 weeks post-BMT, while a 100% survival rate was noted for recipients of control anti-Thyl.2 + C' treated grafts. Weight curves showed that anti-LFA1-RTA pretreatment at the higher concentrations was only marginally protective against GVHD.

The effect of LLME incubation of donor grafts in preventing GVHD induced in C57BL/6 recipients of C3H/HeJ allografts. We next determined if the cells involved in the afferent limb of GVHD in this C3H into C57BL/6 model were distinct from those in the C57BL/6 into B10.BR system. Therefore, we tested BMS treatment with LLME, which like anti-LFA1-RTA will eliminate CTLs, NK cells, and monocytes. We have previously shown that 250 μmol/L LLME pretreat-
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Table 2. Chimerism of Recipients of IT-Treated C57BL/6 BMS Grafts in a GVHD Model

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose (µg)</th>
<th>No. BMT</th>
<th>No. H-2 Typed % Donor* % Host* % Survival</th>
</tr>
</thead>
<tbody>
<tr>
<td>LFA1-RTA</td>
<td>30</td>
<td>20</td>
<td>19</td>
</tr>
<tr>
<td>Thy1.2-RTA</td>
<td>10</td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td>Control-IT</td>
<td>30</td>
<td>20</td>
<td>6</td>
</tr>
</tbody>
</table>

25 x 10^6 BM and 25 x 10^6 splenocytes were treated as described previously and administered to irradiated (8.0 Gy TBI) B10.BR mice.

*Chimerism typing was performed 6 to 8 weeks post-BMT using directly conjugated anti-H-2 and anti-H-2' antibodies.

The effect of anti-LFA1-RTA preincubation of donor grafts on alloengraftment. Because anti-LFA1-RTA was efficacious in preventing GVHD in B10.BR recipients of C57BL/6 allografts, we sought to determine if anti-LFA1-RTA pretreatment of donor grafts had a detrimental effect on alloengraftment. We modified our BMT regimen according to our previous published results and by reducing the TBI dose from 8.0 to 6.0 Gy, deleting the additional splenocytes, and reducing the BM cell number to 18 x 10^6. Recipients were administered BM and spleen cells (25 x 10^6 of each) that were preincubated for 2 hours with anti-LFA1-RTA or a control-RTA IT at the concentrations indicated. The inoculum was incubated with IT in the absence of NH4Cl, thereby resulting in binding without cytolysis. Two additional groups received grafts incubated with C' only or anti-Thy1.2 + C'. Ten mice per group were transplanted. Weight curves are shown.
phils in recipients of anti-LFA1-RTA treated grafts were also decreased at this time post-BMT. They were less than 40% of values observed in recipients of either anti-Thy1.2 + C' or control IT treated grafts. Hematocrit values were equivalently and significantly (P < .05) decreased in recipients of anti-LFA1-RTA or anti-Thy1.2 + C' treated grafts 14 days post-BMT as compared with recipients of control IT treated grafts. Therefore, these data indicate that in the C3H/HeJ into C57BL/6 model, anti-LFA1 pretreatment interferes with donor cell engraftment.

The effect of IT pretreatment or anti-Thy1.2 + C' depletion on bone BM derived day 12 CFU-S formation. Because data in both engraftment systems were consistent with an adverse effect of anti-LFA1-RTA pretreatment on alloengraftment, we examined the effect of IT on day 12 CFU-S formation, an indicator of early progenitor cells contained within the BM graft. Anti-LFA1-RTA (30 µg/mL) significantly (P < .01) reduced the number of day 12 CFU-S, as compared with recipients of anti-Thy1.2 + C' treated, control IT treated (30 µg/mL), anti-Ly1-RTA (30 µg/mL), or LLME (250 µmol/L) treated syngeneic BM cells (Table 4). These data show that in addition to its effect on immune cells, anti-LFA1-RTA pretreatment also reduces the number of early BM progenitor cells.

**DISCUSSION**

Our understanding of the afferent cells involved in GVHD is limited in part by our inability to adequately remove certain types of BM cells in vitro. The use of antibodies that bind to a cell surface epitope provides an important strategy for the selective removal of cell types which may be involved in GVHD generation. In vitro, antibody-mediated cellular elimination is most often accomplished by incubating the target cell populations with antibody followed by C', which provides a means of lysing cells. However, not all antibodies efficiently bind C'. This is the case for FD441.8, a rat antimouse LFA1 α chain MoAb, which inefficiently depletes in vivo. Panning with antibody-coated plates is an alternative strategy, but two or more panning steps are usually required, and a large amount of MoAb is needed for large-scale depletions. Therefore, we conjugated FD441.8 to RTA. Once internalized, RTA catalytically inhibits ribosomal protein synthesis with high efficiency such that a molecule of RTA will inactivate 1,500 ribosomes/minute. With the hybrid molecule, specificity of the IT is conferred by the antibody moiety which is necessary for efficient internalization.

We were interested in the contribution of LFA1 α chain...
expressing cells to the afferent limb of GVHD because antibodies against LFA1 cell surface molecules block T-cell cytotoxicity, NK lysis, and T-cell proliferation, 17-19 each of which has been implicated as playing a role in GVHD. 37 By eliminating cytolytic cell populations, we reasoned that the absence of mature lymphocytes that are transferred in the donor graft and involved in GVHD would prevent GVHD. In the C57BL/6 into B10.BR model, this was the case. In our first-time study of the effect of ex vivo LFA1 depletion on GVHD onset, complete protection against GVHD was observed when grafts were preincubated with only 10 μg/mL of anti–LFA1-RTA in the presence of the potentiation NH₄Cl. A brief (2 hours) preincubation of C57BL/6 BMS grafts with anti-LFA1-RTA was highly effective, enhancing survival rates 80% to 95% as compared with 0% to 20% in recipients of PBS-treated or control IT-treated grafts. Because as few as 5 × 10⁶ splenocytes or 10⁶ C57BL/6 LN T cells infused with BM cells resulted in only a 10% to 30% survival rate, it is likely that 80% or more of the cells involved in the afferent GVHD response were eliminated. The action of the IT was selective because control IT did not protect against GVHD.

An interesting finding was that the complete anti-GVHD effect induced by anti–LFA1-RTA treatment in the C57BL/6 into B10.BR model was not observed in the C3H/HeJ into C57BL/6 model. It has been shown that different phenotypic cell populations are more prevalent in the induction of GVHD in recipients of class I only, class II only, minor antigen only, or class I + II + multiple antigen disparate donor grafts. CD4⁺ cells mediate GVHD in congenic models expressing only class II differences and CD8⁺ cells mediate GVHD in congenic models expressing only class I differences. 38 However, responses to minor antigens are always mediated by CD8⁺ T cells, although in some cases, CD4⁺ T cells are also involved. 39-41

Thus, cell types involved in GVHD induction may depend on the donor/recipient strain combinations chosen. In the strain combination C57BL/6 into B10.BR, the donor and recipient differ in the H-2 region and the minor antigen H-9 (which can serve as a sufficient disparity for the induction of GVHD at high T-cell doses). 41 In this combination, the type of target antigens that dominate the afferent limb of GVHD response in vivo appears to require the involvement of LFA1⁺ donor cells. In the C3H/HeJ into C57BL/6 system, a number of different minor antigenic disparities (not including H-9) exist (H-1, -3, -7, -8, -12, -13, T1a, M1s). Therefore, classical LFA1⁺ or LLME sensitive donor cytolytic cells may not be involved in these responses. For example, Parkman 42 has described autoreactive clones isolated from mice with acute GVHD, which are CD4⁺ and noncytolytic, as well as a minority of CD8⁺ noncytolytic clones with antirecipient restricted minor histocompatibility antigen specificities.

Other explanations for the disparity in the two models are possible. Soluble mediators produced by CD4⁺ (or CD8⁺) cells, such as interferon β or interferon γ, which have been implicated in minor antigen disparity induced chronic GVHD, 43 may be involved in some acute GVHD.

| Table 3. Chimerism After Pretreatment of Donor Grafts With Anti–LFA1-RTA in C57BL/6 Into B10.BR or C3H/HeJ Into C57BL/6 Allotransplants |
|-----------------|-----------------|-----------------|-----------------|-----------------|
| Treatment       | Dose (μg)       | No. BMT         | No. H-2 Typod*  | % Donor         | % Host          |
| C57BL/6 into B10.BR† |                 |                 |                 |                 |                 |
| LFA1-RTA        | 30              | 15              | 9               | 75 ± 12†        | 21 ± 10†        |
| Control-IT      | 30              | 15              | 14              | 99 ± 0          | 0 ± 0           |
| Thy + C'        | NA              | 15              | 15              | 74 ± 6†         | 18 ± 6†         |
| C3H/HeJ into C57BL/6|                 |                 |                 |                 |                 |
| Thy + C'        | NA              | 15              | 12              | 10 ± 7†         | 83 ± 7†         |
| LFA1-RTA        | 30              | 15              | 12              | 0 ± 0           | 93 ± 1†         |
| Control-IT      | 30              | 15              | 15              | 67 ± 11         | 26 ± 9          |

*Chimerism studies were performed 6 to 8 weeks post-BMT using fluorochrome-labeled anti-H-2⁺ and anti-H-2⁻ MoAbs and flow cytometry.
†C57BL/6 BM (18 × 10⁶) was pretreated and administered to irradiated (6.0 Gy TBI) B10.BR.
‡P ≤ .02 as compared with control IT.
§P ≤ .001 as compared with control IT.
[C3H/HeJ BM (18 × 10⁶) was pretreated and administered to irradiated (6.5 Gy TBI) C57BL/6.
because fewer T cells (less) mediate 100% lethality in the because previous studies show that LLME depletion im-

 processes but not others. It is also possible that in some strain combinations, soluble mediators of tissue damage such as tumor necrosis factor may be produced by host macrophages dependent on donor T lymphocytes (conceiv-

able LFA1) for activation. In addition, it is possible that although LFA1 and LLME sensitive cells were equivalently depleted in the BMS inocula of both the C3H/HeJ and the C57BL/6 strains, the lack of efficient GVHD prevention in the recipients of C3H/HeJ BMS may be because fewer T cells (10^6) mediate 100% lethality in the C3H/HeJ system than in the C57BL/6 system. Therefore, the few remaining residual cells in the C3H/HeJ were sufficient to cause GVHD. We do not favor this hypothesis because previous studies show that LLME depletion im-

pressively reduces CTLp (greater than 5 logs). Moreover, C3H/HeJ and C57BL/6 have equivalent sensitivity to LLME (D. Thiele, personal communication). The inferior anti-GVHD effect of LLME depletion in a fully allogeneic model with multiple minor antigen disparities as compared with class I + class II + limited minor antigen disparities supports the hypothesis that the cell types involved in the two different models are distinct.

Because anti–LFA1-RTA could function by either tar-

tected cytotoxicity or blocking LFA1 epitopes and interfer-

ing with the adhesion events in lysis, we tested the IT under conditions in which binding but not lysis was optim-

ized, that is by the omission of NH4Cl. Without NH4Cl, anti–LFA1-RTA was not effective in GVHD prevention, demonstrating that lysis was required for GVHD protec-

tion.

We tested the effects of anti–LFA1-RTA in an alloen- 

graftment model because some other ex vivo TCD strate-

gies in mice (eg, anti-Thy1.2 + C') that prevent GVHD have done so at the expense of engraftment. It was possible that anti–LFA1-RTA pretreatment would not adversely affect allograftment, because LLME pretreatment did not interfere with allograftment in this same model in previous studies. However, the clinical use of LLME may be somewhat problematic due to its documented toxicity to committed human progenitor cells. We do not know whether the α chain of LFA1 was expressed on pluripotent

BM stem cells, but engraftment was not compromised in our GVHD model. In previous studies, doses of anti-LFA1 IT were selected that do not affect human progenitor cells in CFU-GEMM assays in vitro, but still inhibit T-cell function. Additionally, investigators have used in vivo injections of anti-LFA1 to successfully promote engraftment in mice and humans. In these situations, blockade of the host antidonor alloreactive cells is likely occurring and probably impairs T-cell expansion, inhibiting the host-versus-graft effect. Moreover, because in vivo the anti-

LFA1 α chain antibody does not deplete efficiently, access-

ory cells that facilitate alloengraftment may be preserved, as presumably would be the capacity of progenitor cells to yield progeny. This situation does not exist with anti–LFA1-RTA pretreatment in vitro because the IT depletes rather than blocks LFA1 expressing cells. In addition, the cell surface expression of a determinant on stem cells does not necessarily preclude its use for targeting. For example, Thy-1 is expressed on murine stem cells. Despite its expression, BM pretreatment with anti-Thy1.2 IT combined with high-dose TBI conditioning regimens resulted in 100% donor engraftment in mismatched recipients.

In our studies, anti–LFA1-RTA pretreatment significa-

tly decreased long-term allograftment compared to pan-T cell depletion with anti-Thy1.2 + C'. In addition, anti–LFA1-RTA pretreatment reduced hematopoietic re-

covery to a more significant degree for a longer time as compared with anti-Thy1.2 + C' pretreatment. We performed day 12 CFU-S assays and observed an impressive reduction in CFU-S formation in recipients of anti–LFA1-RTA treated, but not anti–Ly1-RTA treated, LLME treated, control IT treated, or anti-Thy1.2 + C' treated syngeneic BM grafts. These data suggest that anti–LFA1-RTA pre-

treatment removed critical accessory cells preserved by anti-Thy1.2 + C', or alternatively, reduced the number of early progenitor cells more efficiently than anti-Thy1.2 + C'. While day 12 CFU-S formation is not necessarily a direct reflection of stem cell repopulation capacity, BM cells giving rise to day 12 CFU-S cells are early progenitor cells. Therefore, a decrement in the number of early committed progenitor cells will likely slow early post-BMT hematopoietic recovery.

In conclusion, anti–LFA1-RTA pretreatment of donor grafts is an effective means of preventing murine GVHD against some, but not all, of the cells that are involved in the induction of murine GVHD. Its potential may be limited by the expression of the α chain of LFA1 on accessory cells or on stem cells responsible for allograftment. Yet, anti-

LFA1-RTA may still be useful for clinical GVHD preven-

tion when combined with positive selection techniques such as elutriation designed to enrich for stem cells.

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REFERENCES

31. Vallera DA, Soderling CCB, Orosz CG: Assessment of immunocompetence by limiting dilution analysis in long-term T
cell depletion chimeras transplanted across the MHC barrier. Transplantation 40:311, 1985
Prevention of murine graft-versus-host disease and bone marrow alloengraftment across the major histocompatibility barrier after donor graft preincubation with anti-LFA1 immunotoxin

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