Importance of minor histocompatibility antigen expression by nonhematopoietic tissues in a CD4+ T cell–mediated graft-versus-host disease model

Stephen C. Jones, … , Thea M. Friedman, Robert Korngold


Minor histocompatibility antigens with expression restricted to the recipient hematopoietic compartment represent prospective immunological targets for graft-versus-leukemia therapy. It remains unclear, however, whether donor T cell recognition of these hematopoietically derived minor histocompatibility antigens will induce significant graft-versus-host disease (GVHD). Using established bone marrow irradiation chimeras across the multiple minor histocompatibility antigen–disparate, C57BL/6→BALB.B combination, we studied the occurrence of lethal GVHD mediated by CD4+ T cells in recipient mice expressing only hematopoietically derived alloantigens. Even substantial dosages of donor C57BL/6 CD4+ T cells were unable to elicit lethal GVHD when transplanted into [BALB.B→C57BL/6] chimeras. Instead, chimeric mice displayed transient cachexia with reduced target-tissue injury over time, reflecting an early, limited, graft-versus-host response. On the other hand, the importance of minor histocompatibility antigens derived from nonhematopoietic tissues was demonstrated by the finding that [C57BL/6→BALB.B] chimeric mice succumbed to C57BL/6 CD4+ T cell–mediated GVHD. These data suggest that severe acute CD4+ T cell–mediated GVHD across this minor histocompatibility antigen barrier depends on the expression of nonhematopoietically rather than hematopoietically derived alloantigens for maximal target-tissue infiltration and injury.

Find the latest version:
https://jci.me/19427/pdf
Importance of minor histocompatibility antigen expression by nonhematopoietic tissues in a CD4+ T cell–mediated graft-versus-host disease model

Stephen C. Jones,1 George F. Murphy,2 Thea M. Friedman,1 and Robert Korngold1

1The Kimmel Cancer Center, and
2Department of Pathology, Jefferson Medical College, Philadelphia, Pennsylvania, USA

Minor histocompatibility antigens with expression restricted to the recipient hematopoietic compartment represent prospective immunological targets for graft-versus-leukemia therapy. It remains unclear, however, whether donor T cell recognition of these hematopoietically derived minor histocompatibility antigens will induce significant graft-versus-host disease (GVHD). Using established bone marrow irradiation chimeras across the multiple minor histocompatibility antigen–disparate, C57BL/6 → BALB.B combination, we studied the occurrence of lethal GVHD mediated by CD4+ T cells in recipient mice expressing only hematopoietically derived alloantigens. Even substantial dosages of donor C57BL/6 CD4+ T cells were unable to elicit lethal GVHD when transplanted into [BALB.B → C57BL/6] chimeras. Instead, chimeric mice displayed transient cachexia with reduced target-tissue injury over time, reflecting an early, limited, graft-versus-host response. On the other hand, the importance of minor histocompatibility antigens derived from nonhematopoietic tissues was demonstrated by the finding that [C57BL/6 → BALB.B] chimeric mice succumbed to C57BL/6 CD4+ T cell–mediated GVHD. These data suggest that severe acute CD4+ T cell–mediated GVHD across this minor histocompatibility antigen barrier depends on the expression of nonhematopoietically rather than hematopoietically derived alloantigens for maximal target-tissue infiltration and injury. J. Clin. Invest. 112:1880–1886 (2003). doi:10.1172/JCI200319427.

Received for publication July 8, 2003, and accepted in revised form September 23, 2003.

Address correspondence to: Robert Korngold, Kimmel Cancer Center, Jefferson Medical College, 233 South Tenth Street, Philadelphia, Pennsylvania 19107, USA. Phone: (215) 503-4552; Fax: (215) 923-4153; E-mail: R.Korngold@mail.jci.tju.edu.

Conflict of interest: The authors have declared that no conflict of interest exists.

Nonstandard abbreviations used: graft-versus-host disease (GVHD); hematopoietic cell transplantation (HCT); bone marrow (BM); minor histocompatibility antigen (miHA); graft-versus-leukemia (GVL); C57BL/6NCR (B6); antibody–T cell–depleted BM (ATBM); mid-survival time (MST); linear millimeter(s) (Lmm).
lethal GVHD (8). Surprisingly, however, in murine BM chimeric recipients with allogeneic MHC class I and II expression restricted exclusively to the hematopoietic compartment, Teshima et al. (9) demonstrated that both CD8+ and CD4+ T cells, respectively, could mediate lethal GVHD. In light of the apparent discrepancy in the role of the hematopoietic compartment in GVHD between these two studies, we have now undertaken to examine the CD4+ T cell responses in a miHA-mismatched BM chimera transplantation model. Here, using the C57BL/6→BALB.B (both H2b) system, we show that hematopoietic expression of miHAs is not enough to drive a CD4-mediated lethal GVHD response, thus implicating nonhematopoietic host cells as critical targets in acute GVHD in a miHA-disparate transplantation setting.

**Methods**

**Mice.** C.B10-H2b/LiMc/J (BALB.B) mice were purchased from The Jackson Laboratory (Bar Harbor, Maine, USA) or raised in our breeding colony from breeder pairs purchased from The Jackson Laboratory. C57BL/6NCR (B6) mice were purchased from the National Cancer Institute Laboratory Animal Production Program (Frederick, Maryland, USA). For all experiments, sex-matched mice between the ages of 6 and 14 weeks were used as donors and recipients. Mice were housed in a pathogen-free environment in autoclaved microisolator cages and were provided with autoclaved water and food ad libitum. All experiments were performed with approval of the Thomas Jefferson University Institutional Animal Care and Use Committee (Philadelphia, Pennsylvania, USA).

**Monoclonal antibodies.** Ascites fluid containing anti-Thy-1.2 (J1j, rat IgM) (10), anti-CD8 (3.168, rat IgM) (11) mAb's were used along with guinea pig complement (C′, Rockland, Boyertown, Pennsylvania, USA) for cell subset depletions. Affinity-purified goat anti-mouse IgG antibody (Cappel-Organon Teknika Corp., West Chester, Pennsylvania, USA) was used for B cell panning. For donor chimerism analysis and phenotypic analysis of donor T cell grafts, FITC- and/or phycoerythrin-labeled mAb’s specific for the following determinants were used: irrelevant isotype control, Ly9.1, CD3e, CD4, CD8e, B220, and CD11c (all from Pharmingen, San Diego, California, USA).

**Flow cytometry.** Between 0.06 and 0.125 μg of each mAb was incubated with 1 × 10⁶ cells or fewer, in PBS containing 1% BSA and 0.01% NaN₃ (wash buffer) for 25 minutes at 4°C. Following multiple washes in wash buffer, either fluorescence analysis was performed immediately or cells were fixed in 1% paraformaldehyde (Electron Microscopy Sciences, Fort Washington, Pennsylvania, USA) for 15 minutes at 4°C, followed by a final wash. Fluorescence analysis was performed on a Beckman Coulter XL-MCL analytic cytometer (Beckman Coulter Inc., Miami, Florida, USA). Flow cytometric gates were established by exclusion of background isotype-control antibody binding.

Of two-color staining, individual populations of control cells were stained with positive-control phycoerythin- and FITC-labeled antibodies, respectively, to assure no background overlap in fluorescence emission between the two fluorochromes.

**Preparation of donor cells.** PBS (BioWhittaker Inc., Walkersville, Maryland, USA) supplemented with 0.1% BSA (Sigma-Aldrich, St. Louis, Missouri, USA) was used for all preparative manipulations of the donor BM and lymphocytes. Antibody–T cell–depleted BM (ATBM) was prepared by flushing of BM cells from the femurs and tibiae of donor mice, followed by incubation with J1j mAb (1:50 dilution) and C′ (1:12 dilution) for 45 minutes at 37°C. T cell–enriched donor cells were prepared from pooled spleen and lymph node cell suspensions from donor mice as previously described (12). Briefly, following red blood cell lysis with Gey’s balanced salt solution containing 0.7% NH₄Cl, B cells were removed by panning of the cell suspension over goat anti-mouse IgG–coated plastic Petri dishes for 1 hour at 4°C. Nonadherent lymphocytes were depleted of CD8+ T cells by incubation with 3.168 mAb (1:50 dilution) and C′ (1:12 dilution) for 45 minutes at 37°C. Donor T cells were more than 97% enriched for the CD4+ subset, as determined by flow cytometry. Cells were injected i.v. in PBS alone.

**Preparation of BM chimeras.** BM chimeras were prepared by transfer of 1 × 10⁶ ATBM cells i.v. into mice exposed 4–6 hours earlier to 13 Gy, split dose (6.5 Gy at 1.36 Gy/min), from a 137Cs Mark-1 model 68 gamma irradiator (J.L. Shepherd and Associates, San Fernando, California, USA). Mice were allowed to reconstitute for 14–17 weeks, at which time a minimum of three mice of representative weight were sacrificed to determine the percentage of donor hematopoietic chimerism by two-color flow cytometric analysis.

**In vivo analysis for GVHD.** BM transplantation was performed as previously described, with some modifications (12). At 14–17 weeks after reconstitution, BM chimeric recipients (indicated in brackets) were reirradiated with 10–13 Gy (split dose) and were injected i.v. 4–6 hours later with a B6 donor inoculum of 2 × 10⁶ ATBM cells, in combination with 1.5 × 10⁶ to 2 × 10⁶ CD4+ T cells. Animals were monitored for morbidity and mortality and weighed regularly until the termination of the experiment. Mid-survival times (MSTs) were calculated as the interpolated 50% survival point of a linear regression through all of the death data points, including zero. Statistical comparisons between experimental groups were based on individual days of death and were performed by the nonparametric Wilcoxon signed rank test. Weight data were expressed as the mean percentage of initial body weight during sequential 1-week periods following transplantation. Significance for weight comparisons between groups was determined by the Student’s t test at particular time points.

**Histology.** Sequential ear biopsies were collected from mice at specific time points, or upon sacrifice of the animal at the conclusion of the experiment, and prepared for routine histological analysis, as previously described.
evance of miHA expression by host hematopoietic or nonhematopoietic tissues to the development of lethal GVHD across this multiple-miHA barrier, [BALB.B→B6] BM chimeric recipients were established such that the only source of alloantigen was the hematopoietic compartment. To this end, B6 mice were lethally irradiated (13 Gy, split dose) and injected with $1 \times 10^7$ BALB.B ATBM cells. BALB.B chimerism was determined 3–4 months later by cytometric staining of splenocytes for the Ly9.1 cell-surface glycoprotein, a lymphoid marker detected on more than 98% of spleen and lymph node cells (16), with differential expression between BALB.B (Ly9.1+) and B6 (Ly9.1−) strains of mice. Within the representative [BALB.B→B6] chimeras analyzed, at least 87% of the T cell compartment and at least 98% of the B cell compartment were BALB.B-derived, as measured by anti-CD3 and anti-B220 staining, respectively. Most importantly, more than 90% of large, CD11c+ cells were BALB.B-derived, likely representing DCs of lymphoid origin (17–19).

Following confirmation of BALB.B hematopoietic engraftment, [BALB.B→B6] BM chimeras were reirradiated (10 Gy, split dose) and injected with $2 \times 10^6$ B6 ATBM cells plus $1.5 \times 10^7$ B6 CD4+ T cells. Idenitically transplanted [BALB.B→BALB.B] and [B6→B6] BM chimeras were used as GVHD positive and negative controls, respectively. All [BALB.B→BALB.B] chimeric recipients succumbed to acute GVHD, with an MST of 26.2 days, whereas the [B6→B6] CD4+ recipients experienced no GVHD lethality (MST >75 days; Figure 1a; Table 1). Importantly, the injection of $1.5 \times 10^7$ B6 CD4+ T cells into [BALB.B→B6] chimeric recipients did not result in GVHD lethality (MST >75 days). Transient cachexia was noted in this group, however ($P < 0.024$ vs. [B6→B6] syngeneic control; Figure 1b), during weeks 2 and 3 after HCT, indicative of an initial B6 CD4+ T cell response against miHAs expressed by the BALB.B hematopoietic compartment. A replicate experiment using identical irradiation doses, but with the injection of $2 \times 10^6$ B6 CD4+ T cells, likewise failed to produce lethality in the [BALB.B→B6] chimeras (MST >90 days; Table 1). As

### Results

In the B6→BALB.B lethal-irradiation GVHD model, donor and recipient mice differ from one another by at least 29 different miHA loci (15). To determine the relationship between miHA expression by host hematopoietic or nonhematopoietic tissues to the development of lethal GVHD across this multiple-miHA barrier, [BALB.B→B6] BM chimeric recipients were established such that the only source of alloantigen was the hematopoietic compartment. To this end, B6 mice were lethally irradiated (13 Gy, split dose) and injected with $1 \times 10^7$ BALB.B ATBM cells. BALB.B chimerism was determined 3–4 months later by cytometric staining of splenocytes for the Ly9.1 cell-surface glycoprotein, a lymphoid marker detected on more than 98% of spleen and lymph node cells (16), with differential expression between BALB.B (Ly9.1+) and B6 (Ly9.1−) strains of mice. Within the representative [BALB.B→B6] chimeras analyzed, at least 87% of the T cell compartment and at least 98% of the B cell compartment were BALB.B-derived, as measured by anti-CD3 and anti-B220 staining, respectively. Most importantly, more than 90% of large, CD11c+ cells were BALB.B-derived, likely representing DCs of lymphoid origin (17–19).

Following confirmation of BALB.B hematopoietic engraftment, [BALB.B→B6] BM chimeras were reirradiated (10 Gy, split dose) and injected with $2 \times 10^6$ B6 ATBM cells plus $1.5 \times 10^7$ B6 CD4+ T cells. Idenitically transplanted [BALB.B→BALB.B] and [B6→B6] BM chimeras were used as GVHD positive and negative controls, respectively. All [BALB.B→BALB.B] chimeric recipients succumbed to acute GVHD, with an MST of 26.2 days, whereas the [B6→B6] CD4+ recipients experienced no GVHD lethality (MST >75 days; Figure 1a; Table 1). Importantly, the injection of $1.5 \times 10^7$ B6 CD4+ T cells into [BALB.B→B6] chimeric recipients did not result in GVHD lethality (MST >75 days). Transient cachexia was noted in this group, however ($P < 0.024$ vs. [B6→B6] syngeneic control; Figure 1b), during weeks 2 and 3 after HCT, indicative of an initial B6 CD4+ T cell response against miHAs expressed by the BALB.B hematopoietic compartment. A replicate experiment using identical irradiation doses, but with the injection of $2 \times 10^6$ B6 CD4+ T cells, likewise failed to produce lethality in the [BALB.B→B6] chimeras (MST >90 days; Table 1). As

### Table 1

Summary of data from transplants with miHAs restricted to the hematopoietic compartment

<table>
<thead>
<tr>
<th>Dose of B6 CD4+ T cells</th>
<th>Reirradiated chimeras Dead/Total MST</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Experiment 1</strong></td>
<td></td>
</tr>
<tr>
<td>$1.5 \times 10^7$ BALB.B → 13 Gy BALB.B</td>
<td>5/5 26.2</td>
</tr>
<tr>
<td>$1.5 \times 10^7$ B6 → 13 Gy B6</td>
<td>0/5 &gt;75</td>
</tr>
<tr>
<td>$1.5 \times 10^7$ BALB.B → 13 Gy B6</td>
<td>0/5 &gt;75</td>
</tr>
<tr>
<td><strong>Experiment 2</strong></td>
<td></td>
</tr>
<tr>
<td>$2 \times 10^7$ BALB.B → 13 Gy BALB.B</td>
<td>3/3 13.3</td>
</tr>
<tr>
<td>$2 \times 10^7$ B6 → 13 Gy B6</td>
<td>0/4 &gt;90</td>
</tr>
<tr>
<td>$2 \times 10^7$ BALB.B → 13 Gy B6</td>
<td>0/5 &gt;90</td>
</tr>
</tbody>
</table>

Chimeras were prepared by transfer of $1 \times 10^7$ ATBM cells into irradiated (13 Gy) mice and then left for 14–17 weeks. Chimeras were then reirradiated (10 Gy) and injected with $1.5 \times 10^7$ to $2 \times 10^7$ B6 CD4+ T cells along with $2 \times 10^6$ B6 ATBM cells.
before, all [BALB.B → BALB.B] recipients succumbed to GVHD (MST 13.3 days), whereas lethality was not noted in the [B6 → B6] group (MST >90 days).

Cutaneous manifestations of acute GVHD are an accurate indicator of clinical disease severity, with the extent of apoptotic injury to skin epidermal cells representing a reliable parameter of disease (14). Ear biopsies harvested on day 8 after HCT from [BALB.B → BALB.B], [BALB.B → B6], and [B6 → B6] chimeric recipients of 1.5 × 10^7 B6 CD4+ T cells revealed similar numbers of dyskeratotic cells, likely reflecting irradiation-related injury at this early time point (P ≥ 0.23, all groups vs. [B6 → B6] recipients; Figure 2d). This early injury was subsequently resolved in [B6 → B6] recipients; however, the level of epidermal apoptosis remained elevated in the skin of [BALB.B → BALB.B] recipients, peaking by day 21 after HCT (Figure 2, a and d). In contrast, in [BALB.B → B6] recipients, the extent of apoptotic injury was similar to [B6 → B6] syngeneic-control levels (P ≥ 0.71, days 21 and 32 vs. [B6 → B6] recipients; Figure 2, b–d).

Despite the absence of acute GVHD in [BALB.B → B6] chimeric recipients, evaluation of ear samples at the conclusion of the experiment (day 90) revealed dermal sclerosis consistent with chronic GVHD in this group (20).

To determine the potential of nonhematopoietically derived miHAs to elicit lethal GVHD, [B6 → BALB.B] BM chimeras were established by injection of lethally irradiated (13 Gy, split dose) BALB.B mice with 1 × 10^7 B6 ATBM cells. Following 3–5 months of hematopoietic reconstitution, the nearly sole source of allogeneic BALB.B miHAs was radiosensitive tissues, such as the

**Figure 2**
Reduced epithelial injury over time in [BALB.B → B6] chimeric recipients. (a–c) Representative histopathological changes in ear biopsies taken on day 21 after HCT. Note the hyperplastic epithelial cell layer and epidermal cell apoptosis (arrows) in skin from [BALB.B → BALB.B] recipients (a), compared with normal-looking skin from [BALB.B → B6] and [B6 → B6] recipients (b and c, respectively). Original magnification: ×200. (d) Sequentially analyzed dyskeratotic index following HCT. Apoptotic-cell counts were taken from more than 10 Lmm of epidermis per sample. Four animals were analyzed per group for all time points, except that only two animals from the [BALB.B → BALB.B] group were analyzed on days 21 and 32; SEM could not be calculated for these latter values (asterisk), but their mean was more than five times the SEM of the experimental and control groups.

**Figure 3**
Minor histocompatibility antigen expression by the hematopoietic compartment results in cutaneous chronic GVHD. (a–d) Sclerotic dermis of skin from [BALB.B → B6] chimeric recipients, harvested day 90 after HCT, was distinguished by thickened dermis (vertical line, a) containing randomly oriented and tightly compacted bundles of collagen (c). In contrast, skin from [B6 → B6] syngeneic controls had a normal dermal thickness (vertical line, b) and contained mostly horizontally oriented, loosely packed bundles of collagen (d). Original magnification: a and b, ×200; c and d, ×400. (e) Mean dermal thickness ± SEM, in millimeters. n = 5 for both groups.
addition, direct MHC class II loading of cytosolic miHAs for presentation by BALB.B APCs, an endogenous pathway that has been demonstrated in other systems (21, 22), might also have occurred. In either case, the data presented here suggest that multiple miHAs expressed by hematopoietic tissues alone is insufficient to drive fully developed lethal, CD4+ T cell–mediated GVHD in the B6→BALB.B model.

The observations made here agree with previous studies involving the CD8+ T cell–mediated B10.BR→CBA miHA-disparate GVHD model. Expression of recipient CBA miHAs exclusively by cells of the hematopoietic compartment in CBA→B10.BR chimeras significantly diminished the development of B10.BR CD8+ T cell–mediated GVHD (8). Moreover, Shlomchik et al. recently utilized high-level irradiation BM chimeras in the CD8-mediated C3H.SW→B6 GVHD miHA-disparate strain combination and found that C3H.SW CD8+ T cells were unable to mediate disease in C3H.SW→B6 chimeras. This suggested that neither donor nor host DC cross-presentation of miHAs alone played a significant role in the pathogenesis of CD8-mediated lethal GVHD (4). However, the role of cross-presentation may still depend on the immunogenicity of the antigens being presented in the particular strain combination (23).

Collectively, the current results are in sharp contrast to the work of Teshima et al., who demonstrated in the MHC class II-disparate bm12→B6→B6.MHC II+/- BM chimeric model that alloantigen expression by the hematopoietic compartment alone was sufficient to obtain lethal GVHD (9). Clearly there are differences in the allogeneic T cell response directed across MHC versus miHA barriers, most notably the involvement of a much higher alloreactive CD4+ T cell precursor frequency in the former situation (24, 25). In bm12→B6 (9) and similar models (26–30), such a vigorous T cell response can rapidly generate high levels of proinflammatory cytokines responsible for initiating acute tissue damage in the early post-HCT period. Early injury to the gut epithelium allows for the transmission of LPS into the systemic circulation and peripheral tissues, with subsequent activation of macrophages, and release of high levels IL-1 and TNF-α (31, 32). Both of these inflammatory cytokines have established roles in the pathogenesis of lethal GVHD (26, 30, 33–37). The finding that MHC class II expression by host nonhematopoietic target tissues in the B6→B6.MHC II+/- chimera was not essential for the cascade of inflammatory events that ultimately lead to GVHD and lethality (9) suggested that, in such models, either large amounts of systemic cytokines alone are sufficient to cause severe tissue injury, or resident allogeneic B6 APCs in the tissues are adequate to activate infiltrating T cells to mediate injury via localized cytokine production. In this model, allogeneic B6 APCs would still be able to process and present MHC class II molecules bearing antigens derived from their own self-proteins, but even more so from the surrounding nonhematopoietic cells of B6 origin, which merely lack MHC class II expres-
The nonhematopoietic tissue may thus serve as a major source of miHAs for processing and presentation by APCs to infiltrating CD4+ T cells. Furthermore, studies demonstrating the expression of MHC class II molecules by keratinocytes and gut epithelial cells following allogeneic HCT (43–46) suggest that miHAs expressed by nonhematopoietic tissues may be directly presented to allogeneic CD4+ T cells. In addition, data demonstrating reduced GVHD severity in models utilizing either FasL-deficient (47, 48) or perforin-deficient (49) CD4+ T cells support a role for CD4+ T cells as cytolytic effectors capable of cell-to-cell contact and antigen-dependent cytotoxicity following allogeneic HCT. The importance of miHAs expressed by nonhematopoietic tissues in inducing acute GVHD was emphasized by the finding of lethal GVHD following injection of B6 CD4+ T cells into [B6→BALB.B] chimeras. In this case, GVHD would seem to be dependent on nonhematopoietic miHAs processed and presented by donor-type B6 APCs, by the few residual BALB.B-type APCs, or by upregulated class II molecules on BALB.B epithelial cells themselves. Further studies are needed to delineate the role of each of these presentation pathways.

Although there was minimal evidence of acute GVHD in the skin of [BALB.B→B6] chimeric recipients, by day 90 at the conclusion of the experiment, a significant increase in the deposition of thick collagen bundles in the dermis was observed, consistent with scleroderma-like chronic GVHD (20). Chronic GVHD in patients may develop without being preceded by acute GVHD, lending support to the hypothesis that the pathogenesis of the two conditions is significantly different. Our observation in the dermis could relate to a fundamental difference in target-cell types responsible for acute versus chronic GVHD. While acute disease may involve both cytokine-mediated and cytolytic epithelial injury, chronic GVHD may depend primarily on T cell interaction with hematopoietically derived APCs and the subsequent release of cytokines. Potential target cells in the skin include long-lived BM-derived dermal DCs that possess potent antigen-presenting activity and could thereby provide a constant source of allogeneic stimulation for chronic GVHD (50).

In conclusion, in the B6→BALB.B model studied here, donor CD4+ T cell-alloreresponses directed against multiple-miHa differences derived exclusively from the recipient hematopoietic compartment results in a limited form of GVHD characterized by early, transient cachexia, with minimal lasting tissue injury. In contrast, T cell responses directed against miHAs derived from nonhematopoietic tissues result in severe, lethal GVHD. Ultimately, studies investigating the identity of GVHD-causing class II–restricted miHAs will permit elucidation of their tissue-expression patterns and thus facilitate an understanding of their individual role in development of GVHD immunopathology.

**Acknowledgments**

This work was supported by NIH grants HL55593 and CA40358.


