ibid. **19**, 320 (1994); H. H. H. Göring and J. D. Terwilliger, in preparation.

18. Pedigree members who tested positive for the disease in either the clinical or the radiological test were coded as affected, and those testing negative in both tests were coded as unaffected. Individuals who were only examined by one method (clinical or radiological) and were found to be normal were coded as having unknown phenotypic status. Unexamined individuals, including married-in spouses and deceased individuals from earlier generations, were likewise coded as unknown in their phenotype.
19. $Z_1 = \log[L(\hat{\vartheta})/L(\vartheta = 0.5)]$ is a test for linkage. [$L(\vartheta)$ denotes the likelihood as a function of the recombination fraction ϑ.] The allele frequencies of the observed polymorphism were treated as nuisance parameters using "profile" likelihoods [R. Royall, *Statistical Evidence: A Likelihood Paradigm* (Chapman & Hall, London, 1997), pp. 158–161]; that is, the likelihood was maximized over these allele frequencies independently under the null hypothesis of no linkage and under the alternative hypothesis of linkage [(*25*), pp. 186–187; H. H. H. Göring and J. D. Terwilliger, in preparation]. A lod score of 3 is generally considered to be significant [N. E. Morton, *Am. J. Hum. Genet.* **7**, 277 (1955)] and asymptotically corresponds to a pointwise *P* value of 0.0001 [J. Chotai, *Ann. Hum. Genet.* **48**, 359 (1984)].
20. Affecteds-only analysis provides only little evidence for linkage (lod score 0.5), whereas the linkage disequilibrium results conditional on linkage remain significant (lod score 6.3) (data not shown). It is not that surprising, however, that the linkage lod score is reduced, because much linkage information is provided by unaffected individuals when the disease is dominant with high penetrance. To avoid spurious results, we only coded pedigree members as unaffected when they were found to be normal in two different diagnostic schemes, as mentioned above.
21. $Z_2 = \log[L(\hat{\hat{\vartheta}}, \hat{\hat{\delta}})/L(\hat{\vartheta}, \delta = 0)]$ is a test for linkage disequilibrium conditional on linkage. δ here denotes linkage disequilibrium. In the denominator [identical to the numerator of Z_1 (*20*), only written differently], the likelihood is maximized over the marker allele frequencies using ILINK, as before. In the numerator, the likelihood is maximized over the disease-marker haplotype frequencies, keeping the marginal frequency of the disease-predisposing allele constant. This maximization was performed with the EH program [(*25*), pp. 200–203] using only unrelated individuals, as ILINK would have changed the marginal disease-allele frequency also. Although the haplotype frequencies estimated with the EH program may not be the maximum likelihood estimates, this procedure is conservative in that the true maximum likelihood estimates are used in the denominator. This lod score has two degrees of freedom. (There are five free parameters in the numerator: ϑ and four haplotype frequencies, the frequencies of the two remaining haplotypes being constrained by the marginal allele frequencies of the disease locus. There are three free parameters in the denominator: ϑ and two allele frequencies, with the frequency of the remaining allele being constrained because the allele frequencies need to sum to 1.) For interpretation, the *P* value for the observed lod score is given in the text.
22. The evidence for linkage disequilibrium conditional on linkage using the whole data set (families and unrelated singletons) is much higher than if the case and control data had been analyzed separately. Using Fisher's "exact" test to test for equality of Trp allele frequencies among cases (6 of 314 chromosomes) and controls (0 of 348 chromosomes) only gives a *P* value of about 0.01. The reason for this apparent discrepancy is that, in joint linkage and linkage disequilibrium analysis, the linkage disequilibrium provides not only phase information for pedigree founders, but also genotype information for untyped founders. For this reason, joint analysis of linkage and linkage disequilibrium is often much more than the sum of the parts, sometimes leading to much higher lod scores (*15*, *17*).
23. $Z_3 = \log[L(\vartheta = 0)/L(\vartheta = 0.5)]$ is a test of complete linkage. In the denominator, the likelihood was maximized over the allele frequencies of the polymorphism. In the numerator, however, the frequency of the Trp allele was fixed to be equal to the frequency of the disease-predisposing allele (because we wanted to test whether the Trp allele itself is a disease-causing allele), and the likelihood was maximized over the remaining allele frequencies of the polymorphism. $Z_4 = \log[L(\vartheta = 0, \delta = \text{complete})/L(\vartheta = 0, \delta = 0)]$ is a test of complete linkage disequilibrium conditional on complete linkage. In the denominator (identical to the numerator in Z_3, only written differently), the frequency of the Trp allele was set to be equal to the frequency of the disease allele and the likelihood maximized over the remaining marker allele frequencies. In the numerator, complete linkage disequilibrium was assumed. In other words, all Trp chromosomes carry the disease allele and vice versa, and all non-Trp chromosomes carry the "normal" allele at the disease locus and vice versa. The marginal frequency of the haplotype with the disease-predisposing allele was kept constant, and the likelihood was maximized over the remaining haplotype frequencies using ILINK (*16*).
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Prevention of Graft Versus Host Disease by Inactivation of Host Antigen-Presenting Cells

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Graft versus host disease, an alloimmune attack on host tissues mounted by donor T cells, is the most important toxicity of allogeneic bone marrow transplantation. The mechanism by which allogeneic T cells are initially stimulated is unknown. In a murine allogeneic bone marrow transplantation model it was found that, despite the presence of numerous donor antigen-presenting cells, only host-derived antigen-presenting cells initiated graft versus host disease. Thus, strategies for preventing graft versus host disease could be developed that are based on inactivating host antigen-presenting cells. Such strategies could expand the safety and application of allogeneic bone marrow transplantation in treatment of common genetic and neoplastic diseases.

Allogeneic bone marrow transplantation (alloBMT) has revolutionized the treatment of hematopoietic malignancies, inherited hematopoietic disorders, and aplastic anemia. Unfortunately, graft versus host disease (GVHD) remains a major toxicity that greatly limits the application and efficacy of alloBMT (*1*). Most patients who undergo alloBMT receive stem cells from major histocompatibility complex (MHC)–identical donors. In these patients, GVHD is initiated by donor T cells that recognize a subset of host peptides, called minor histocompatibility antigens (miHAs), which are derived from the expression of polymorphic genes that distinguish host from donor (*2*). Presently, therapy for GVHD is limited to immunosuppression directed largely against T cells (*3*).

T cell responses are initiated on antigen-presenting cells (APCs). In alloBMT the unusual situation arises in which both host- and donor-derived APCs are present. We therefore examined the roles of host- and donor-derived APCs in initiating GVHD with the goal of identifying another target for GVHD prevention (*4*–*6*). Because recipient hematopoiesis is ablated by cytotoxic therapies before the transplant, and more intensive radiation augments GVHD, donor APCs might be crucial (*7*). On the other hand, peptides presented to $CD8^+$ T cells by MHC class I molecules are derived primarily from endog-

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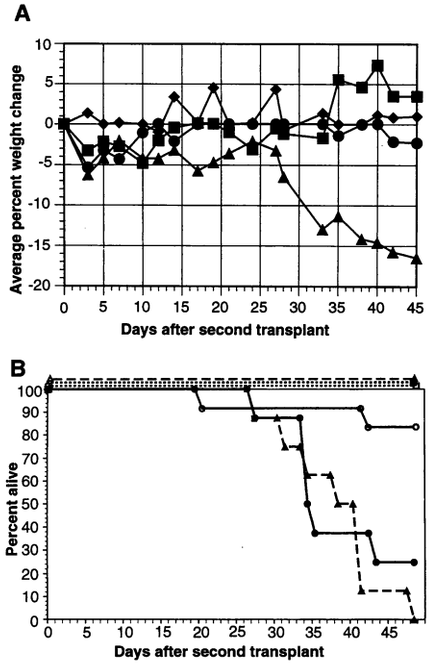

Fig. 1. (**A**) Percent weight loss. Average percent weight change versus time is plotted. Groups are (squares) B6→B6 recipients of C3H.SW T^{depl}BM; (circles) $\beta_2M^{-/-}$→B6 recipients of C3H.SW T^{depl}BM; (triangles) B6→B6 recipients of C3H.SW T^{depl}BM plus 10^6 $CD8^+$ T cells; and (diamonds) $\beta_2M^{-/-}$→B6 recipients of C3H.SW T^{depl}BM plus 10^6 $CD8^+$ cells. (**B**) Survival. $\beta_2M^{-/-}$→B6 and B6→B6 chimeras were reirradiated and received C3H.SW T^{depl}BM with or without purified C3H.SW $CD8^+$ T cells. Groups are (closed squares) B6→B6 recipients of T^{depl}BM; (closed circles) B6→B6 recipients of T^{depl}BM plus 10^6 $CD8^+$ cells; (closed triangles) B6→B6 recipients of T^{depl}BM plus 2×10^6 $CD8^+$ cells; (open squares) $\beta_2M^{-/-}$→B6 recipients of T^{depl}BM; (open circles) $\beta_2M^{-/-}$→B6 recipients of T^{depl}BM plus 10^6 $CD8^+$ cells; and (open triangles) $\beta_2M^{-/-}$→B6 recipients of T^{depl}BM plus 2×10^6 $CD8^+$ cells.

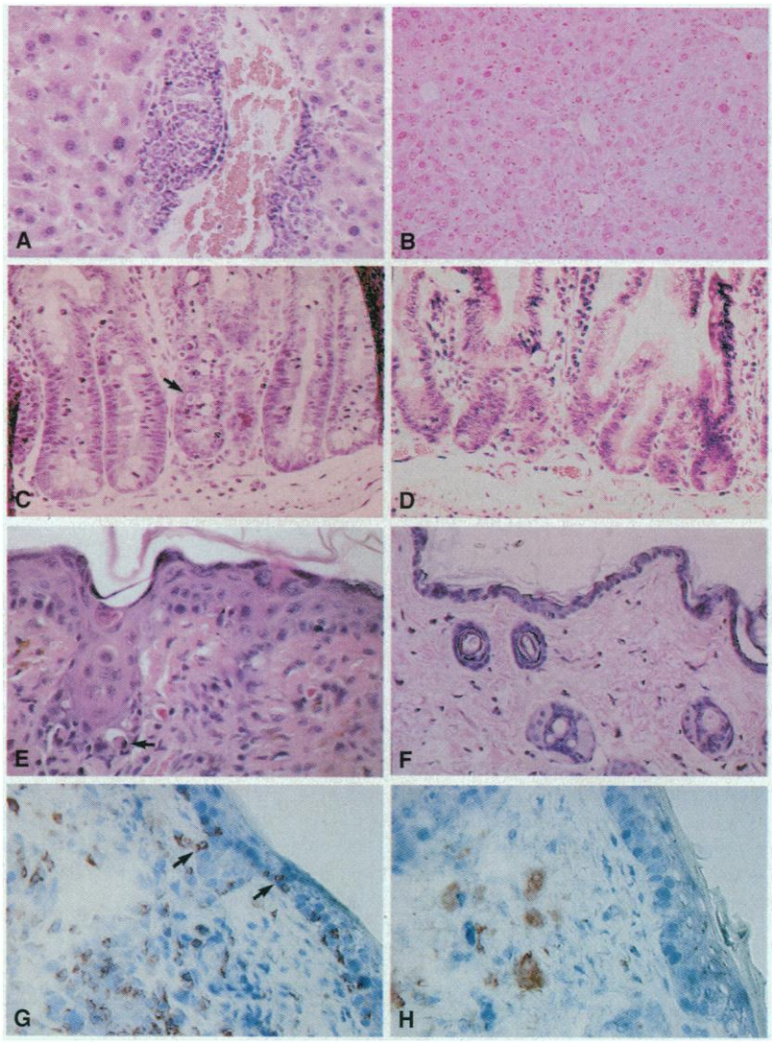

Fig. 2. $\beta_2M^{-/-}$→B6 bone marrow chimeras are resistant to GVHD induction. Histology from $\beta_2M^{-/-}$→B6 (**B**, **D**, and **F**) and B6→B6 (**A**, **C**, **E**, **G**, and **H**) recipients of C3H.SW T^{depl}BM and $CD8^+$ T cells are shown: (A and B) liver, (C and D) small intestine, and (E and F) skin. Periportal mononuclear infiltrates are present in (A); apoptotic cells are found in small bowel crypts in (C) (arrow); and mononuclear cell infiltrate, fibrosis, epidermal maturation disarray, and necrotic keratinocytes (arrow) are visible in (E). These changes were absent in $\beta_2M^{-/-}$→B6 recipients. (G) Horseradish peroxidase immunohistochemistry for $CD8^+$ cells. Note $CD8^+$ cells invading follicles (lower left) and epidermis (arrows). (H) Immunohistochemical staining for $CD4^+$ cells from the same mouse as in (G). Note the absence of $CD4^+$ cells in epidermis. Diffuse red staining in the dermis is background. (For all panels, 1 mm = 6.5 μm.)

enously expressed genes (*8*), so host APCs might be essential. However, extracellular antigens can be "cross-presented" on class I, thereby "cross-priming" donor T cells (*9, 10*).

Using a MHC-identical, multiple miHA mismatched murine model of alloBMT analogous to most human alloBMTs, we examined whether host mice with APCs unable to present class I–restricted peptides would develop GVHD. We generated bone marrow chimeric mice without class I on their APCs but with class I on target tissues (*11*). Wild-type C57BL/6 (B6; H-2^b) hosts received 1000 cGy of ionizing radiation to eradicate host hematopoiesis, including APCs, followed by reconstitution with T cell–depleted bone marrow (T^{depl}BM) from B6 β_2-microglobulin knock out mice ($\beta_2M^{-/-}$). $\beta_2M^{-/-}$ cells do not express class I and therefore cannot present peptide antigens to $CD8^+$ T cells (*12*). After waiting 4 months for $\beta_2M^{-/-}$ hematopoietic engraftment and APC repopulation, we used these chimeras (designated $\beta_2M^{-/-}$→B6) as recipients in a GVHD-inducing alloBMT (*11*). The chimeras were reirradiated and then injected with T cell–depleted C3H.SW (H-2^b) bone marrow (C3H.SW T^{depl}BM) (*13*) with or without 10^6

Table 1. Histologic scoring of GVHD. Formalin-fixed, paraffin-embedded sections were stained with hematoxylin and eosin, randomized, and read blindly by experienced pathologists. Findings were scored and given an overall interpretation of positive (+), indefinite (+/−), or negative (−) for GVHD. *N*, number of mice analyzed.

Group	*N*	Liver			Intestine			Tongue			Ear			Skin		
		+	+/−	−	+	+/−	−	+	+/−	−	+	+/−	−	+	+/−	−
B6→B6 BM/CD8	6	4	2	0	4	1	1	6	0	0	6	0	0	5	0	1
$\beta_2M^{-/-}$→B6 BM/CD8	6	0	1	5	0	0	6	0	1	5	1	1	4	0	0	6
B6→B6 BM	3	0	2	1	0	2	1	0	0	3	0	0	3	0	0	3
$\beta_2M^{-/-}$→B6 BM	3	0	0	3	0	0	3	0	1	2	0	0	3	0	0	3

or 2×10^6 purified C3H.SW $CD8^+$ T cells (*14*). As controls, B6→B6 syngeneic chimeras were treated identically.

In each of three experiments, the $\beta_2M^{-/-}$→B6 recipients of C3H.SW $T^{depl}BM$ plus $CD8^+$ T cells were resistant to GVHD induction. In contrast, the B6→B6 recipients of C3H.SW $T^{depl}BM$ plus $CD8^+$ cells developed severe GVHD manifested by hunched posture, skin and ear erythema, alopecia, weight loss (Fig. 1A), and death. Only 1 of 30 tissue samples from $\beta_2M^{-/-}$→B6 CD8 recipients showed evidence of GVHD, in contrast to 25 of 30 tissue samples from B6→B6 CD8 recipients (Fig. 2 and Table 1). $CD8^+$ but not $CD4^+$ T cells were detected in skin lesions, confirming the pathogenic role of $CD8^+$ T cells (Fig. 2, G and H).

In a second experiment, $\beta_2M^{-/-}$→B6 and B6→B6 chimeras received C3H.SW $CD8^+$ cells and were monitored for survival (Fig. 1B). Six of eight B6→B6 recipients of 10^6 C3H.SW $CD8^+$ cells and 8 of 8 recipients of 2×10^6 $CD8^+$ cells died with clinical GVHD, whereas only two deaths occurred in the 24 $\beta_2M^{-/-}$→B6 chimeric recipients of C3H.SW $CD8^+$ cells ($P = 0.0024$, Fisher's exact test; comparison between all B6→B6 and $\beta_2M^{-/-}$→B6 $CD8^+$ T cell recipients). In a third experiment in which all mice received 2×10^6 $CD8^+$ T cells, GVHD was again inhibited in the $\beta_2M^{-/-}$→B6 C3H.SW CD8 recipients. However, in this case, delayed (40% longer mean time to onset) and less severe (46% less mean weight loss) GVHD was observed among 3 of 8 mice compared with 7 of 8 B6→B6 C3H.SW CD8 recipients.

The finding of milder and delayed GVHD among 4 of 38 $\beta_2M^{-/-}$→B6 $CD8^+$ T cell recipients over three experiments suggested that either replacement of host APCs with $\beta_2M^{-/-}$ APCs was variably incomplete, or that in a minority of mice, donor-derived APCs cross-primed donor T cells. To quantitate host APC replacement, we analyzed spleen and lymph node cells from $\beta_2M^{-/-}$→B6 chimeras by flow cytometry before the second transplant (Fig. 3). The range of residual host dendritic cells (0.2 to 17.8%) suggests that "breakthrough" GVHD was due to variable APC turnover and that complete depletion of class I^+ APCs may not be required for protection from GVHD. The greater degree of host macrophage persistence (3 to 30%) suggests that they may not be important APCs in these experiments.

The failure of the $\beta_2M^{-/-}$→B6 recipients of C3H.SW $CD8^+$ T cells to develop GVHD was not due to rejection of donor $CD8^+$ T cells or bone marrow. Using antibodies specific for an allelic form of the T cell marker CD5 expressed on C3H.SW but not on B6 T cells, we observed C3H.SW $CD8^+$ T cells in the $\beta_2M^{-/-}$→B6 chimeras. Nearly all of the $CD11b^+$ and $CD11c^+$ cells in the $\beta_2M^{-/-}$→B6 chimeras that underwent the second transplant were class I^+, confirming donor C3H.SW APC engraftment (*15*).

Although cross-priming occurs in a variety of experimental situations (*9, 10*), in the $\beta_2M^{-/-}$→B6 chimeras described here, cross-presentation of host antigens by donor APCs was insufficient to generate GVHD. Therefore, either host APCs are more efficient than donor APCs in cross-presenting exogenous antigens, cross-presentation does not induce GVHD, or both. To test whether host-derived APCs could cross-present exogenous antigens, we next used C3H.SW→B6 bone marrow chimeras as recipients for an alloBMT with C3H.SW $T^{depl}BM$ and $CD8^+$ T cells. For these mice to develop GVHD, resident C3H.SW APCs would need to cross-present B6 antigens. However, such C3H.SW→B6 chimeras were completely resistant to GVHD (Fig. 4). Thus, when APCs are limited to the cross-presentation of exogenous antigens, they cannot induce clinical GVHD even when resident in a chimeric host for a long period. Although dendritic cells can efficiently cross-present antigens from apoptotic cells (*10*), which are in abundance after irradiation, this was insufficient to induce GVHD. Therefore, either cross-presentation is inefficient or apoptotic bodies are rapidly cleared by other mechanisms, thereby limiting their exposure to dendritic cells. These data also suggest that initial target antigens for $CD8^+$ T cells in GVHD are restricted to proteins expressed by host APCs.

Suppressor cells have been proposed as mediators of resistance to GVHD (*6, 16*), but appear unlikely to explain the present data. The simplest explanation for the resistance of both the $\beta_2M^{-/-}$→B6 and the C3H.SW→B6 chimeras is the absence of APCs capable of presenting host antigens via the endogenous pathway. If suppressor cells were responsible for the absence of GVHD, they

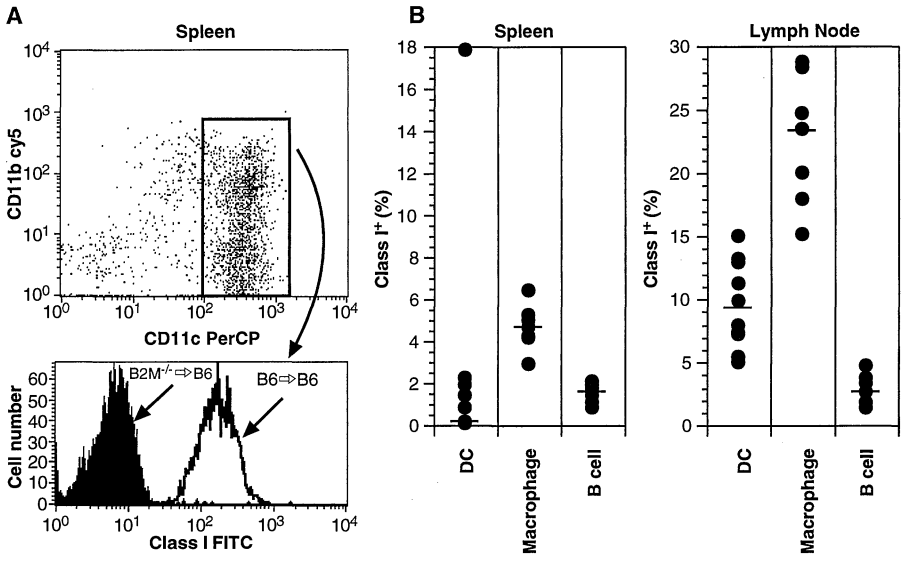

Fig. 3. Class I expression on dendritic cells, macrophages, and B cells. (**A**) Class I expression by dendritic cells. Dendritic cells were isolated by digesting spleens and lymph nodes with collagenase, followed by centrifugation through 30% bovine serum albumin. Dendritic cells were identified by four-color flow cytometry. Cells staining with a multilineage mixture of phycoerythrin-conjugated antibodies to Thy1.2 (T cells), Gr-1 (granulocytes), TERR 119 (erythroid), and CD45R (B220; B cells) were excluded. Class I expression on $CD11c^+$ cells is shown. FITC, fluorescein isothiocyanate; PerCP, peridinin chlorophyll protein. (**B**) Class I expression by dendritic cells (DC), macrophages, and B cells in lymph nodes and spleens of $\beta_2M^{-/-}$→B6 chimeras. Circles, individual mouse; dashes, median. Twelve mice were analyzed for splenic and lymph node dendritic cell chimerism; seven mice were analyzed for macrophage and B cell chimerism.

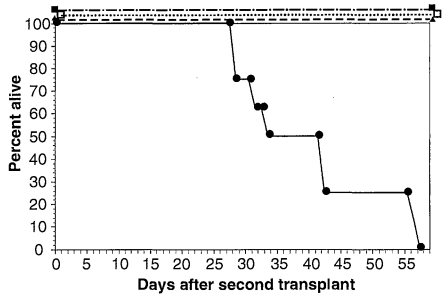

Fig. 4. C3H.SW→B6 bone marrow chimeras are resistant to GVHD induction. B6 mice received two 500-cGy fractions then were reconstituted with 7×10^6 C3H.SW or B6 $T^{depl}BM$. Four months later, the chimeras were reirradiated with two 375-cGy fractions and received C3H.SW $T^{depl}BM$ with or without 2×10^6 C3H.SW $CD8^+$ T cells. Groups are (triangles) B6→B6 recipients of C3H.SW $T^{depl}BM$; (circles) B6→B6 recipients of C3H.SW $T^{depl}BM$ plus $CD8^+$ T cells; (closed squares) C3H.SW→B6 recipients of C3H.SW $T^{depl}BM$; and (open squares) C3H.SW→B6 recipients of C3H.SW $T^{depl}BM$ plus C3H.SW $CD8^+$ T cells.

would have to have developed only in the $\beta_2M^{-/-}$→B6 and C3H.SW→B6 chimeras, and not in the B6→B6 controls. In any event, if suppressor cells were involved, host APCs would be required for their development.

These results contrast with those of Sprent and colleagues (*4*). They found that in a $CD8^+$ T cell–dependent MHC-compatible but miHA-incompatible GVHD model donor→host bone marrow chimeras that were reirradiated and injected with donor bone marrow and lymph node cells developed GVHD. They suggested that either the chimeras were not devoid of host APCs because of insufficient radiation, or that host antigens were processed by donor marrow cells. Our data support the former explanation. Sprent and colleagues also found that heavily irradiated allogeneic parent→F_1 bone marrow chimeras developed GVHD in response to very high doses of parental T cells (up to 8×10^7 T cells per recipient) and concluded that nonhematopoietic cells functioned as APCs. Given the increased precursor frequency of T cells recognizing allogeneic MHC molecules in comparison to miHA on self MHC and the large dose of T cells used, they may have unmasked the presence of small numbers of residual host APCs. Alternatively, nonhematopoietic cells when challenged with very large numbers of allogeneic T cells may cause T cell activation sufficient to induce GVHD.

Our results suggest that depleting host APCs before the conditioning regimen should abrogate GVHD without the need for prolonged T cell–targeted immunosuppression. Such an approach, perhaps using toxin-conjugated or radiolabeled antibodies, could expand the range of diseases treated with alloBMT. To test the feasibility of in vivo antibody-mediated depletion of host dendritic cells, we injected mice with N418, a hamster monoclonal antibody to the β integrin CD11c expressed on murine dendritic cells (*17*). The injected anti-CD11c bound to all CD11c-expressing dendritic cells in both lymph node and spleen (*15*), supporting the feasibility of antibody-mediated APC depletion.

A subset of alloBMT recipients have self-limited GVHD, which was presumed to reflect acquired T cell tolerance. Our data suggest another explanation: replacement of host with donor APCs abrogates T cell activation. Infusions of T cells from original bone marrow donors given to relapsed leukemia patients months to years after the initial alloBMT (*18*) cause less GVHD than has been observed when T cells are given at the time of transplantation (*19*). Although there may be other explanations (*20*), we suggest that the replacement of host with donor APCs reduces the chance of a donor $CD8^+$ T cell interacting with a GVHD-inducing host APC. In addition to suggesting explanations for these clinical observations, our data provide the foundation for a different strategy for reducing GVHD-host APC depletion. This approach may avoid the problems associated with T cell depletion of marrow allografts: failure of engraftment, poor immune reconstitution, and lack of immunoreactivity against the tumor.

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11. B6 mice received 200 μg of antibody to NK1.1 (anti-NK1.1) on consecutive days (*21*) followed by two 500-cGy fractions and injection with 10^7 $T^{depl}BM$ cells from B6 $\beta2M^{-/-}$ or B6 mice. Four months later, chimeras were reirradiated with two 375-cGy fractions, then injected with 7×10^6 C3H.SW $T^{depl}BM$ cells with or without 10^6 or 2×10^6 C3H.SW $CD8^+$ T cells.
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13. T cells were depleted with anti-Thy1.2–labeled magnetic microbeads (Miltenyi Biotec) according to the manufacturer's protocol.
14. $CD8^+$ T cells were isolated by staining lymph node cells with biotin-labeled antibodies to CD4, B220, and CD11b, followed by depletion with streptavidin-conjugated magnetic beads (Miltenyi Biotech). Ninety-five percent of cells were $CD8^+$; less than 0.25% of cells were $CD4^+$.
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Generation of a Widespread *Drosophila* Inversion by a Transposable Element

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Although polymorphic inversions in *Drosophila* are very common, the origin of these chromosomal rearrangements is unclear. The breakpoints of the cosmopolitan inversion *2j* of *D. buzzatii* were cloned and sequenced. Both breakpoints contain large insertions corresponding to a transposable element. It appears that the two pairs of target site duplications generated upon insertion were exchanged during the inversion event, and that the inversion arose by ectopic recombination between two copies of the transposon that were in opposite orientations. This is apparently the mechanism by which transposable elements generate natural inversions in *Drosophila*.

Most *Drosophila* species are naturally polymorphic for inversions in one or more chromosomes (*1, 2*), but little is known about the molecular mechanisms underlying the generation of these inversions. Indirect evidence suggests that transposable elements (TEs) cause *Drosophila* inversions: TEs mediated chromosomal rearrangements in laboratory populations (*3*), and in situ hybridization studies detected the transposon *hobo* around the breakpoints of four endemic inversions of *D. melanogaster* (*4*). However, in *D. subobscura* and *D. pseudoobscura* there was no cytological association between middle repetitive sequences and inversion breakpoints (*1, 2*), and the two direct studies that sequenced the breakpoints of naturally occurring inversions did not detect any TE (*5*).

The species *D. buzzatii* belongs to the *D. repleta* group of the *Drosophila* subgenus (*6*). Two chromosomal arrangements are commonly observed in chromosome *2* of *D. buzzatii*: the ancestral one or *2 standard* (*2st*), and the *2j*, which derived from the *2st* by inversion *2j* and is distributed throughout the species range at high frequencies (*7*). Here, we cloned and sequenced the breakpoints of inversion *2j* of *D. buzzatii*. First, the region of the proximal breakpoint in the *2st* chromosome (designated as

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