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Rapid Reconstitution of Antibody Responses Following Transplantation of Purified Allogeneic Hematopoietic Stem Cells

Jessica A. Linderman and Judith A. Shizuru

Allogeneic hematopoietic cell transplantation has broad clinical applications extending from the treatment of malignancies to induction of immunologic tolerance. However, adaptive cellular and humoral immunity frequently remain impaired posttransplantation. Here, recovery of T-dependent and T-independent Ab responses was evaluated in mice transplanted with purified hematopoietic stem cells (HSCs) devoid of the mature immune cells believed to hasten immune recovery. Mixed and full donor chimeras were created by conditioning recipients with sublethal or lethal irradiation, respectively, across different donor/host genetic disparities. By 6 wk posttransplantation, all animals demonstrated robust T-dependent Ab responses equivalent to those of untransplanted controls. Full chimeras that received fully MHC-disparate HSCs showed delayed T-dependent Ab responses that recovered by 12 wk. This delay occurred despite early reconstitution and proper migration to germinal centers of donor-derived T follicular helper (TFH) cells. Congenic transplants into TFH-deficient CD4^-/- mice revealed restoration of T-dependent Ab responses by 6 wk, leading us to conclude that MHC disparity caused delay in humoral recovery. These findings, together with our previous studies, show that, contrary to the view that depletion of graft lymphocytes results in poor posttransplant immunity, elimination of immune-suppressing graft-versus-host reactions permits superior immune reconstitution. This study also provides insight into the regeneration of TFH cells and humoral immunity after allogeneic HSC transplantation. The Journal of Immunology, 2011, 186: 000–000.

The most readily available pool of organ donors is imperfectly matched at the HLA complex, and such HLA disparities place patients at high risk for severe GVHD if mature donor immune cells are present within the graft. Transplantations that combine non-myeloablative treatments and manipulated grafts result in the establishment of mixed hematopoietic chimerism that is sufficient to achieve a tolerant state. Despite these advances in graft manipulation and conditioning regimen, impairment of adaptive immunity has remained a concern for all patients who undergo allogeneic hematopoietic cell transplantation. Most data on this topic come from patients that receive standard transplantations using myeloablative conditioning and infusion of unmanipulated allografts. In these recipients, lymphoid reconstitution is compromised, and T-dependent humoral immunity is significantly impaired for a minimum of 6 mo after transplantation (4). It is argued that mature lymphocytes contained in an allograft could contribute to the restoration of adaptive immunity, and this perception is supported by clinical studies showing that reduction of graft T cells leads to a significantly higher rates of opportunistic fungal and viral infections (5). However, clinical data focusing on recipients of the most rigorously T cell-depleted grafts report no delay in immune reconstitution compared with that of patients receiving T cell-replete grafts (6). We and others (7–13) have shown in preclinical mouse studies that, rather than enhancing immune recovery, mature postthymic T cells can exert deleterious GVHRs resulting in quantitative reduction in lymph node size and lymphocyte number and qualitative reduction in T cell proliferative responses to nominal Ag. These manifestations of GVHR were observed in recipients that received relatively low numbers of T cells and did not display overt signs of GVHD. Thus, given the risk for GVHR even without clinical GVHD, transplantation of highly purified hematopoietic stem cell (HSC)
grafts may result in superior immune recovery by eliminating the immune suppression associated with GVHRs.

In this study, immune reconstitution following purified allogeneic hematopoietic stem cell transplantation (HSCT) was assessed by measuring Ab responses to T-dependent and T-independent Ags. Clinical studies report a general deficiency in Ab production after allogeneic transplantation (14–16), and preclinical studies have shown this impairment to be particularly pronounced in response to T-dependent Ags after fully MHC-disparate transplantation. In fully MHC-disparate mice prepared with either myeloablative (10, 17) or non-myeloablative (18) conditioning, marked delay or failure of donor B cells to produce Abs to T-dependent Ags has been observed, respectively. This impaired Ab production is not surprising given the complex MHC-restriction requirements of CD4+ T cell development and the downstream interactions between T and B cells. Specifically, following fully MHC-disparate transplantation, the MHC type expressed on donor B cells differs from that expressed by radiation-resistant host thymic stromal cells. As the MHC expressed on cortical thymic epithelium plays a critical role in CD4+ T cell selection and development, most T cells that exit the thymus of a hematopoietic chimera will be restricted to host-type MHC and not the donor-type MHC expressed on donor B cells in the periphery.

Our studies aimed to determine the causes of Ab deficiency after MHC-disparate HSCT with a particular focus on B cell and T follicular helper (TFH) cell function and restriction. TFH cells are a distinct subset of Ag-experienced CD4+ T cells with the highest ability to induce B cell Ab production (19). Although TFH cell development is not fully understood (20), it is known that TFH differentiation requires either sustained periods of Ag exposure or high-affinity peptide–MHC/TCR interactions (21) and thus may be a rate-limiting factor in the recovery of Ab responses posttransplantation. In this study, we examined Ab responses in transplantations of MHC-matched, haploidentical, and fully MHC-disparate purified HSC grafts in myeloablative and non-myeloablative recipients. We observed prompt recovery of Ab responses to both T-dependent and T-independent Ags when donor and recipients shared an MHC haplotype. In fully MHC-disparate chimeras, donor-derived T-dependent Ab responses were delayed despite early functional reconstitution of donor-derived TFH cells but recovered by 12 wk post-HSCT.

Materials and Methods

Mice

All mice were bred and maintained at the Stanford University Research Animal Facility under strict pathogen-free conditions. As shown in Table I, hematopoietic cell donors were 6–8-wk-old Thy-1.1, CD45.1 congenic C57BL/Ka (H-2b) mice for the fully MHC-disparate and MHC-matched transplant. Donors for the haploidential transplantations were generated by breeding SWR (H-2d, Thy-1.2, CD45.2) with C56BL/Ka-Thy1.1-CD45.1 mice to produce F1(C57BL/Ka-Thy1.1-CD45.1 × SWR) mice (H-2b/d). Recipients were 8–12-wk-old BALB.B (H-2d, Thy1.2, CD45.2) mice for the MHC-matched and BALB/c (H-2b, Thy1.2, CD45.2) mice for fully MHC-disparate transplant. For the haploidential transplantation experiments, SWR mice were bred with BALB/c mice to generate F1(SWR/J × BALB/c) (H-2a/d). CD4+/-animals were B6.129S2-Cd45.1<sup>m<sup>Missouri/I and purchased from The Jackson Laboratory.

HSC purification and hematopoietic cell transplantation

Purified HSCs were obtained by a modified procedure originally described by Spangrude et al. (22). Bone marrow cells were harvested from the femurs of donor mice and positively selected for c-Kit surface expression using c-Kit mAb microbeads and MidiMACS separation units (Miltenyi Biotec, Auburn, CA). c-Kit-positive cells were then stained for a second epitope of c-Kit using an aliphophycocyanin-conjugated mAb 2B8, FITC-conjugated Thy1.1, PE-Cy7-conjugated Sca-1, and lineage markers (CD3, CD4, CD8, CD6, B220, Gr1, Mac1, and Ter119) conjugated to PE. Cells were sorted by multiparameter flow cytometry with positive selection for c-Kit, Thy1.1, and Sca-1 and negative selection for the lineage markers. The resultant purified population is referred to throughout the text as HSC. Five thousand HSCs were infused by retro-orbital injection within 12 h of irradiation. Lethal radiation doses were delivered to the recipients based on titrations done for each strain. BALB.B and BALB/c mice were given 800 cGy, C57BL6/J and CD4+/- mice were given 950 cGy, and F1(SWR/J × BALB/c) mice were given 1000 cGy whole-body gamma-irradiation, delivered in two split doses >3 h apart. Sublethal doses of irradiation were 75% of the lethal dose for each strain. Fully MHC-disparate and haploidential hosts were pretreated with two doses of 100 mg of the anti-NK cell polyclonal Ab anti-asialo GM1 7d and 1 d before transplant. Chimerism analyses of engraftment were performed using flow cytometry of whole blood based on CD45.1 and CD45.2 differences.

Analysis of Ab production

Mice were immunized at the base of the tail and in the hock with 15 mg of the antigenic protein suspended in a 1:1 (v/v) emulsion of PBS and CFA containing 1 mg/ml Mycobacterium tuberculosis strain H37Rv. On day 9, serum was collected from animals, and an ELISA was performed. Plates were coated with a specific Ag and loaded with serum diluted 1:100 in PBS at 2% Tween 20. The 1:100 dilution was chosen based on the saturation points and detection levels determined by dilution curves of serum from injected control animals. Abs were detected with biotinylated anti-mouse Ig, IgG1, or IgG2a (Southern Biotech) or IgG1<sup>a</sup>, IgG2a<sup>b</sup>, or IgG2a<sup>b</sup> (BD Biosciences). A streptavidin–alkaline phosphatase secondary was added, and plates were read at 405 nm after addition of SIGMAFAST p-nitrophenyl phosphate (Sigma Aldrich).

Analysis of TFH cells

Nine days after immunization, inguinal and popliteal lymph nodes were harvested and made into single-cell suspension. TFH cells were analyzed using flow cytometry using CD4-allophycocyanin-Cy7, FITC-CD44, ICOS-PerCP-Cy5.5, and CD45.1-allophycocyanin (epitope) and CXCR5-PE amplified with anti-PE-biotin and streptavidin PE (BD Biosciences). Live cells were determined using Fixable Aqua Stain Kit, Live/Dead (BD Biosciences).

Immunofluorescence

Inguinal lymph nodes harvested 9 d after immunization were snap frozen in Optimal Cutting Temperature (Tissue-Tek). Five-micrometer-thick sections were cut, and germinal centers were stained with biotinylated peanut agglutinin (Vector) and mouse anti-Thy-1.1 (eBioscience) followed by streptavidin-594 and anti-mouse IgG 488 (Invitrogen). Photomicrographs were obtained using a Leica DM5000B microscope.

Statistical methods

All tests were two-tailed unpaired t tests between individual groups to the 95% significance level. When reported in comparison with two controls, the lower significance level is reported. Statistical analysis was performed using the Prism software package.

Results

Early impairment of Ab responses to T-dependent neoantigens early after fully MHC-disparate transplantations

To evaluate Ab responses after allogeneic HSCT, transplantations were performed between fully MHC-disparate, haploidential, and MHC-matched donors and hosts (Table I). For fully disparate chimeras, purified HSCs from C57BL/Ka-Thy1.1-CD45.2 (H-2b) were transplanted into irradiated BALB/c (H-2b) hosts. Haploidential donors were F1(C57BL/Ka-Thy1.1-CD45.2 × SWR) (H-2b/d) and share the H-2b of all MHC and half of all minor histocompatibility genes with hosts F1(SWR × BALB/c) (H-2b/d). MHC-matched transplant were done between C57BL/Ka-Thy1.1-CD45.2 (H-2b) and BALB.B hosts, which differ at minor histocompatibility loci. Two doses of total body irradiation were used for each pair: lethal irradiation resulting in full chimeras and a sublethal dose resulting in mixed chimerism. Full chimeras had more than 90% donor contributions in the B cell, macrophage, and granulocyte lineages by 6 wk post-HSCT and are referred to as full chimeras despite a significant host contribution to the T cell
lineage. Sublethal irradiation resulted in mixed chimeras defined by T cell, B cell, macrophage, and granulocyte lineage levels in the blood of >15% donor-type and >15% host-type by 6 wk after HSCT.

Chimeric mice were challenged with a novel T-dependent Ag 6 wk after HSCT. We previously determined that full chimeras receiving MHC-matched or haploidentical HSCs have peripheral blood cell counts within the normal range, and full chimeras of all donor/host combinations tested here had robust CD4+ T cell proliferative responses restricted to peptides presented on donor-type MHC by 6 wk post-HSCT (7). Prior to antigenic challenge, we established that the peripheral blood of all animals contained levels of B cells not statistically different from those of untransplanted controls (Supplemental Fig. 1). Animals were then immunized at the base of the tail and in the hock with a haptenated protein, 2,4,6-trinitrophenol OVA (TNP-OVA), emulsified in CFA. Levels of TNP-OVA specific Ab in the serum were measured 9 d later. Fully MHC-disparate full chimeras demonstrated significantly lower levels of Ag-specific Abs in the serum compared with those of untransplanted controls and mixed chimeras (p < 0.0001) (Fig. 1A). In contrast, mixed chimeras that were fully MHC-disparate as well as haploidentical and MHC-matched mixed chimeras had TNP-OVA specific Ab levels that did not differ significantly from those of controls (Fig. 1A–C).

These experiments were repeated using a different Ag, 2,4,6-trinitrophenol keyhole limpet hemocyanin (TKP-KLH), to exclude the possibility that this phenomenon of reduced Ab production in fully MHC-disparate full chimeras was Ag specific (Fig. 1D, 1E). In fully MHC-disparate chimeras, both full and mixed chimeras had uniformly lower Ab responses to TNP-KLH than those of untransplanted controls (p < 0.005) (Fig. 1D). However, robust Ab responses were again noted in haploidentical chimeras that were not statistically different from those of controls (Fig. 1E) suggesting that shared MHC identity between the recipient and responding donor B cell population influenced the Ab response. These studies led us to conclude that reduced Ab production in fully MHC-disparate chimeras is not limited to a particular Ag.

Abs present at 6 wk in fully MHC-disparate mixed chimeras are host-derived

To understand better the nature of the deficiency in Ab production to TNP-OVA, we determined if donor- and/or host-derived B cells were responsible for Ab production in the mixed fully MHC-disparate, haploidentical, and MHC-matched chimeras. Because there is no single-detection Ab able to discriminate all donor Igs from host Igs, we used two sets of allele-specific, isotype-specific detection Abs that could differentiate between donor- and host-derived IgG2a/c and IgG1. Given the similarity of the relative donor and host contributions to the Ag-specific Ab responses within these two isotypes and to the IgM response (data not shown), we believe that analysis of these individual isotypes is reflective of the donor and host contribution to total Ag-specific Ig.

As expected based on total TNP-OVA specific Ab levels, no IgG2a/c was detected in fully MHC-disparate full chimeras (Fig. 2A, 2B). In the fully MHC-disparate mixed chimeras, however, only host-derived IgG2a/c was measurable. This skewing of host B cell-derived Ab production differed markedly in the haploidentical chimeras. In haploidentical full chimeras, all IgG2a/c was donor-derived, and both donor and host B cells contributed to the specific Ab response in mixed chimeras (Fig. 2C, 2D). MHC-matched chimeras showed a trend similar to the haploidentical pairs with measurable donor-derived Ab present in full and mixed chimeras (Fig. 2E) and host-derived Ab in mixed chimeras (Fig. 2F). Measurements of donor and host contributions to Ag-specific

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### Table I. Donor and host characteristics

<table>
<thead>
<tr>
<th>Strain Disparity</th>
<th>Donor Characteristic</th>
<th>Recipient Characteristic</th>
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<tr>
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<td>MHC</td>
<td>Thy-1</td>
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<td>Fully MHC-disparate</td>
<td>C57BL/Ka-Thy1.1-CD45.1</td>
<td>H-2b</td>
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<tr>
<td>Haploidentical</td>
<td>F1[C57BL/Ka-Thy1.1-CD45.1xSWR/J]</td>
<td>H-2h</td>
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<td>C57BL/Ka-Thy1.1-CD45.1</td>
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### FIGURE 1. Ab responses to novel T-dependent Ags 6 wk post-HSCT. Fully MHC-disparate (A, D), haploidentical (B, E), and MHC-matched (C) full and mixed chimeras were challenged with the novel T-dependent Ag TNP-OVA (A–C) or TNP-KLH (D, E) 6 wk after transplant. Serum levels of Ag-specific Ig 9 d after immunization were measured using ELISAs. Results are shown as OD readings with untransplanted controls of donor and host strains. Ab levels were statistically lower than those of untransplanted controls in fully MHC-disparate full chimeras challenged with TNP-OVA (p < 0.0001) and in fully MHC-disparate full and mixed chimeras challenged with TNP-KLH (p < 0.006). For all other conditions, Ab levels were not statistically different from those of untransplanted controls.
IgG1 showed the same trends as IgG2a/c in fully MHC-disparate and MHC-matched chimeras (Supplemental Fig. 2), but shared IgG1 allotypes meant that donor and host origin could not be determined in the haploidentical setting.

Analysis of Ab responses to the alternative Ag TNP-KLH showed a poor IgG2a/c response even in untransplanted controls. However, the more robust IgG1 responses showed a similar trend of no IgG1 in the fully MHC-disparate full chimeras and only host-derived IgG1 in the fully MHC-disparate mixed chimeras (Supplemental Fig. 3).

Rapid reconstitution of Ab responses to a novel T-independent Ag

The above findings suggested that donor-derived B cells are capable of producing Ab at 6 wk post-HSCT, but only when donors and hosts shared an MHC allele. To determine whether the deficiency seen in fully MHC-disparate animals was limited to T-dependent Ags, chimeras were challenged with the T-independent Ag 2,4,6-trinitrophenol Ficoll (TNP-Ficoll). TNP-Ficoll specific Ab levels were not statistically different in fully MHC-disparate full or mixed chimeras compared with those of untransplanted controls (Fig. 3A). Allotype analysis showed that donor-derived Ab dominated the response in full chimeras and that both donor and host B cells produced TNP-Ficoll specific Ab in mixed chimeras (Fig. 3B, 3C). Thus, whereas Ab responses to T-dependent Ags are impaired in fully MHC-disparate chimeras at 6 wk post-HSCT, responses to T-independent Ags are intact at this time point.

Donor-derived T<sub>FH</sub> cells are present at 6 wk post-HSCT

T<sub>FH</sub> cells have been identified as the CD4<sup>+</sup> T cell subset responsible for supporting B cell Ab production to T-dependent Ags. Because the defect in Ab production was specific to donor-derived Ab when no MHC class II alleles were shared between donor and host, we hypothesized that a lack of T<sub>FH</sub> cells restricted to donor MHC class II could explain this finding. In recipients of fully MHC-disparate HSCs, we examined the T<sub>FH</sub> compartment for the presence of donor- versus host-derived T<sub>FH</sub> cells. Although the origins of the T<sub>FH</sub> cells might not strictly correlate with their MHC restriction, the presence of donor-derived T<sub>FH</sub> cells would show that nascent donor T<sub>FH</sub> had developed post-HSCT when donor-type MHC were present to potentially drive selection and differentiation.

T<sub>FH</sub> cells are CD4<sup>+</sup> T cells defined by a composite surface phenotype. CD44<sup>+</sup> expression indicates that these cells have interacted with their cognate Ag, chemokine receptor CXCR5<sup>+</sup> ex-
TFH cells interact with B cells most efficiently within the germinal center during an immune response. T cells migrate to the B cell that of the untransplanted controls (Fig. 4). Allotype analysis showed high levels of donor-derived IgG2a/c in both full and mixed chimeras (Fig. 4A). The majority of the TFH cells in full chimeras were of donor origin (Fig. 4C). In mixed chimeras, the donor contribution to the TFH subset was consistent with the percentage donor contribution to all CD4+ T cells, the latter of which varied between recipients. Thus, it appeared that the origin, donor or host, did not affect the ability of nascent CD4+ cells to differentiate into a TFH cell in these chimeras.

Proper germinal center formation and localization of TFH cells

TNP-Ficoll Specific Antibodies

Donor-Derived IgG2a/c

Host-Derived IgG2a/c

FIGURE 3. T-independent Ab responses 6 wk post-HSCT. Fully MHC-disparate full and mixed chimeras were challenged with TNP-Ficoll 6 wk after transplantation, and serum levels of TNP-Ficoll specific Ab were measured. Transplanted animals show total TNP-specific Ig levels equivalent to those of untransplanted controls of donor and host strains (A). Allotype analysis showed high levels of donor-derived IgG2a/c in both full and mixed chimeras (B) and host-derived Ab responses in mixed chimeras (C).

Ab responses in fully MHC-disparate full chimeras recover by 12 wk post-HSCT

To determine if the Ab deficiency seen in MHC-disparate chimeras was a lasting deficiency or one that improved with time, full chimeras were challenged at time points beyond 6 wk at 9, 12, and 15 wk after transplantation. Levels of TNP-OVA specific Ab after Ag challenge remained significantly lower than those of untransplanted controls at 9 wk post-HSCT ($p = 0.0004$) but recovered by 12 wk (Fig. 6A). Responses to TNP-KLH (Fig. 6B) recovered by 9 wk, even faster than responses to TNP-OVA. This recovery was not due to a relative increase in TFH cells over time as the percentage TFH cells in the lymph nodes of fully MHC-disparate full chimeras showed no significant differences compared with percentages at 6 wk (Supplemental Fig. 4). As expected in full chimeras, the Ab response was donor-derived at 15 wk (Fig. 7A). However, it is interesting to note that only host-derived and not donor-derived TNP-OVA specific Abs were detected in fully MHC-disparate mixed chimeras.

T-dependent Ab responses after transplantation into CD4−/− hosts

Donor-derived Tfh cells were present in the lymph node germinal centers by 6 wk after HSCT. Thus, the lack of a donor-derived B cell response early after transplant in fully MHC-disparate chimeras might be explained by the incongruence between the MHC of the donor and recipient. Specifically, the good responses seen early after transplant when donor and host shared MHC alleles could be due to the persistence of host-derived, host-restricted CD4+ T cells that had developed prior to HSCT and survived radiation. These CD4+ T cells could interact with donor- or host-derived B cells expressing host-type MHC, which were lacking in the fully MHC-disparate pairs, and drive responses in the periphery. To test this hypothesis, we used CD4-deficient mice as recipients. These mice have a genetic disruption within the CD4 gene locus, causing an impediment in CD4+ cell development. Although some cells develop a Th-like phenotype, Ab production to T-dependent Ags is not detected in these mice. CD4 wild-type, CD45 congenic HSCs were transplanted into lethally irradiated hosts.
CD4<sup>−/−</sup> mice. We reasoned that recipients of such transplanta-
tions would reveal the time point at which to expect a restored
T-dependent Ab response driven by the reconstitution of T<sub>FH</sub>
cells derived from the transplanted HSCs, as no remaining host-
derived CD4<sup>+</sup> cells would be present to drive an Ab response.

At 6 wk posttransplantation of congenic HSCs, the CD4<sup>−/−</sup>
recipients were able to mount Ab responses to TNP-OVA signif-
icantly greater than those of untransplanted CD4<sup>−/−</sup> animals and
equivalent to those of wild-type animals receiving congenic
transplants (Fig. 8). These results show that, if no genetic disparity
exists between donor and recipient, T<sub>FH</sub> cells arise from HSCs and
are capable of mediating T and B cell interactions to produce Abs
as early as 6 wk post-HSCT. This rapid recovery of congenic
CD4<sup>−/−</sup> hosts contrasts the recovery of T-dependent Ab responses

FIGURE 4. Donor- and host-derived T<sub>FH</sub>
cells. Lymph nodes harvested 9 d after T-
dependent Ag challenge with TNP-OVA
from fully MHC-disparate and haploidenti-
cal chimeras as well as untransplanted
controls were analyzed for the percentage
of T<sub>FH</sub> (CD4<sup>+</sup>CD44<sup>+</sup>CXCR5<sup>+</sup>ICOS<sup>+</sup>) cells
using the gating strategy shown (A). The
percentage of T<sub>FH</sub> cells was then calculated
for each group and showed no significant
differences between chimeric animals and
untransplanted controls (B). The numbers in
parentheses show the number of animals
used for each condition. At least half of the
T<sub>FH</sub> cells in fully MHC-disparate animals
were of donor origin in full chimeras, and
the donor-derived percentage of T<sub>FH</sub> cells in
mixed chimeras reflected the overall chi-
merism of the CD4<sup>+</sup> T cell lineage (C). The
numbers in parentheses show the number of
animals used for each condition.

FIGURE 5. Donor-derived T<sub>FH</sub> localization to
germinatal centers. Lymph nodes were harvested from
fully MHC-disparate full (A) and mixed (B) chimeras,
haploidentical full (C) and mixed (D) chimeras, and
an untransplanted donor control (E). Sections were
stained with peanut agglutinin (in red) to mark ger-
minatal centers and Thy1.1 (in green) to mark donor-
derived T cells. Small germinal centers formed in all
transplant recipients, and donor-derived T cells were
present in those germinal centers. Mixed chimeras
showed more robust germinal center formation, al-
though haploidentical full chimeras had larger ger-
minal centers than did fully MHC-disparate full
chimeras. Original magnification ×20.
in the fully MHC-disparate chimeras at 6 wk suggesting that the genetic disparity between the donor and host, not solely the time required for T\(_{FH}\) cells to develop from HSCs, plays a prominent role in delaying reconstitution of humoral immunity.

**Discussion**

Infections resulting from immunodeficiency after allogeneic transplantation remain a leading cause of posttransplant morbidity and mortality. Clinical studies report that after standard myeloablative transplants, patients demonstrate impaired B cell recovery with significantly reduced Ab production for at least 6 mo post-transplantation (4). Even in the MHC-matched transplant setting, B cell immunity can be dysfunctional for up to 2 yr posttransplantation (15), and B cell responses are depressed for longer periods in patients with more genetically disparate donors (25).

Clinical data on immunity posttransplantation is complicated by the fact that many patients experience some degree of GVHR, which has known immunosuppressive effects, and by the treatment of GVHD with immunosuppressive drugs. Clinically obvious chronic GVHD, which arises after day 100 posttransplantation, is known to be associated with prolonged and significantly impaired Ab production (4). Studies of mouse hematopoietic cell transplantation models in our laboratory have yielded further evidence that GVHR impairs immune function even when the level of mature cells present in a graft are too low to induce any clinical symptoms of GVHD (7). Rigorously purified HSC grafts lacking mature immune cells can resolve this problem, allowing transplantation without GVHR. Although purified HSCs do not transfer the same graft-versus-tumor activity as grafts replete with postthymic T cells, their use in transplantation for nonmalignant indications such as immune deficiencies, hemoglobinopathies, and tolerance induction would permit transplantation between donors and hosts of greater genetic disparity than is currently feasible.

In this study, we used mouse models to evaluate comparatively Ab responses to novel T-dependent Ags in recipients of purified HSCs that were MHC-matched, haploidentical, or fully MHC-disparate. The robust recovery of Ab responses we observed under all conditions contrasts with the large body of data showing an enduring T-dependent Ab deficiency after unpurified bone marrow transplantation in fully MHC-disparate mice (26). We found that by 12 wk post-HSCT, recipients in all genetic combinations were able to mount Ab responses to T-dependent Ags equivalent to those of untransplanted controls. This recovery of Ab responses to novel T-dependent Ags occurred even earlier, by 6 wk after HSCT, in MHC-matched and haploidentical full chimeras, wherein donor and host shared at least a subset of their MHC alleles. Furthermore, all sublethally irradiated mixed chimeras had similarly robust T-dependent Ab responses at 6 wk post-HSCT, albeit only host B cells contributed to this response in fully MHC-disparate chimeras. We interpret these data to mean that, in the absence of

**FIGURE 6.** Recovery of T-dependent Ab responses in fully MHC-disparate full chimeras. Fully MHC-disparate full chimeras were challenged with TNP-OVA (A) or TNP-KLH (B) at 6, 9, 12, and 15 wk after transplant. Nine days after immunization, serum levels of Ag-specific Ig were measured, and each mark represents an individual animal. TNP-OVA specific serum levels remained significantly lower than those of untransplanted controls at 9 wk (p < 0.0005) but reached levels equivalent to those of untransplanted controls by 12 wk post-HSCT.

**FIGURE 7.** Donor- and host-derived TNP-OVA specific Abs in fully MHC-disparate chimeras at 15 wk posttransplant. Allotype-specific Abs were used to detect the origin, donor or host, of TNP-OVA specific IgG\(_{2a/c}\). Donor-derived (A) but not host-derived (B) IgG\(_{2a/c}\) is present in fully MHC-disparate full chimeras at 15 wk. Only host-derived Ag-specific Ab is detected in mixed chimeras.

**FIGURE 8.** Functionality of donor-derived T\(_{FH}\) cells at 6 wk after transplant. Congenic HSCs were transplanted into CD4\(^{−/−}\) hosts to determine if newly arising donor-derived T cells could drive Ab responses against the T-dependent Ag TNP-OVA. Six weeks after congenic transplantation into CD4\(^{−/−}\) or wild-type (WT), recipients and untransplanted controls were immunized. Each mark represents the total Ig level for an individual animal. Ab responses in CD4\(^{−/−}\) hosts transplanted with HSCs were significantly lower than those of untransplanted controls (p = 0.012) but not significantly different from those of wild-type hosts transplanted with congenic HSCs (p = 0.61).
GVHR, lethally or sublethally irradiated animals can promptly recover B cell function post-HSCT. Further, when MHC identity is partially shared between donor and recipient, sufficient T cell help is present to cooperate with the B cells as early as 6 wk post-HSCT.

Only fully MHC-disparate chimeras showed significant delay in reconstitution of T-dependent responses compared with the other genetic combinations tested. Because we have observed that B cells rapidly repopulate the recipients of MHC-disparate HSCs within weeks following HSCT and that donor-derived T-independent Ab responses recover by 6 wk post-HSCT, we hypothesized that the defect in Ab production in fully MHC-disparate chimeras was due to an inability of T cells to provide help to B cells effectively. Efficient cooperation between T and B cells relies upon selection of developing T cells on the same MHC-haplotype as expressed by the B cell. It is generally accepted that developing T cells are positively selected on radioresistant thymic epithelial cells (27–29). In the case of MHC-disparate HSCT, it is thought that host thymic epithelial cells shape the repertoire of T cells that enter the periphery to interact with donor B cells of a different MHC type. The exact contribution of bone marrow-derived cells to this positive selection process is debated. In mouse models of fully MHC-disparate transplantation, some reports suggest that T cells restricted to donor-type MHC never arise (30), whereas others, using different combinations of donors and hosts, suggest a T cell repertoire develops that can respond to Ags in the context of either donor or host MHC (31).

Previous work in our laboratory examining thymic selection in the same fully MHC-disparate donor and host combination as studied here demonstrated that CD4+ T cells can proliferate in response to donor-MHC restricted peptides by 6 wk post-HSCT (7). In contrast, achievement of donor-MHC restriction within this time period was not seen here for T-dependent Ab responses. Because functional differences may exist between the ability of T cells to proliferate in response to Ag presented on donor-type MHC versus their ability to provide help to B cells expressing donor-type MHC, we examined the development of T FH cells in the lymph nodes of HSCT chimeras. T FH cells have only recently been characterized as a distinct subset that has the greatest ability to induce B cell Ab production (19). The time course of T FH development remains uncertain (20), with our work being the first to examine T FH development after transplantation. In fully MHC-disparate chimeras, we noted that phenotypic T FH cells were present in percentages comparable with those of congenic HSCT recipients, MHC-matched HSCT recipients, and wild-type animals that had donor-derived T FH cells localized in the proper anatomic sites by 6 wk post-HSCT. To test the functionality of donor T FH cells derived from transplanted HSCs, we used CD4-deficient mice as hosts. These mice show a profound deficiency in T cell help of B cells prior to transplantation, thus we reasoned that any T FH cell function observed after transplant would be of donor origin. When CD4-deficient mice were transplanted with congenic HSCs and challenged 6 wk post-HSCT, measurable responses to a T-dependent Ag were noted. This result suggests that T FH cells derived from donor HSCs are functional at the 6-wk time point and able to drive T-dependent Ab responses.

Given that donor-derived T FH cells are functional at 6 wk postcongeneric HSCT, we conclude that the genetic difference between donor and recipient delayed the appearance of donor-restricted T FH cells capable of conferring help to donor B cells. Our data here and elsewhere (7) support the idea that in the early post-HSCT phase, the CD4+ cells capable of driving Ab responses to T-dependent Ags adhere more strictly to the need for shared MHC identity between thymic stroma and APCs in the periphery compared with CD4+ cells generally able to proliferate in response to nominal Ag. We hypothesize that this deficiency in fully MHC-disparate pairs is due to the high-affinity interactions required for cells to differentiate into T FH cells. Affinity appears to be crucial for development of functional T FH cells. Upon antigenic challenge, T cells with the highest affinity for peptide–MHC remain in the B cell area for the longest time and preferentially differentiate into T FH cells (21). Using transgenic T cells of varying affinity, it was shown that T cells with higher-affinity TCRs that were able to interact for longer periods of time with Ag being presented by B cells produced more IL-21 and higher levels of Bcl-6, both of which are important hallmarks of T FH cells. The absence of shared MHC alleles between donor and host results in donor-restricted T cells that are low in number, have a limited repertoire selected either on the basis of cross-reactivity or by a selecting element derived from HSC, and/or have a lower affinity for Ags than host-restricted T cells at 6 wk post-HSCT. Over time, a more extensive donor-restricted T cell repertoire is established that includes increasing numbers of T cell clones of higher affinity for peptide presented on donor-type MHC, likely accounting for functional donor-restricted immune responses being restored. Future studies using adoptive transfer of T cells with transgenic TCRs specific for Ag presented by donor-type MHC should more definitively test this hypothesis.

This host versus donor differential affinity hypothesis explains several observations: 1) the lower numbers of T cells present in the peripheral blood and lymph nodes 6 wk post-HSCT in full chimeras receiving fully MHC-disparate grafts than those in animals receiving HSCs from MHC-identical donors (7); 2) the delay in T-dependent Ab responses in recipients of fully MHC-disparate compared with MHC-matched donors or CD4+/− recipients of congenic HSC; and 3) the observation in mixed MHC-disparate chimeras that donor-derived Ab is absent as late as 15 wk post-HSCT. We postulate that, even weeks after transplant, more host-restricted T cells selected by the host-type MHC of the thymus will be of high affinity compared with the population of donor-restricted T cells that enter the periphery. In full chimeras, these high-affinity host-restricted cells are unable to drive B cell responses because the lymph nodes are populated by only donor B cells. However, in mixed chimeras, host-restricted T cells can interact with host B cells. These high-affinity interactions then induce IL-21 production, resulting in expansion of host B cells and ultimately in host B cells dominating the germinal center reaction and Ab production.

In summary, our data show that early and robust Ab responses to novel T-dependent Ags can be achieved after transplantation of purified allogeneic HSCs in the absence of GVHRs if donor and recipient share MHC alleles. Further, although there appear to be stringent requirements for MHC identity in the selection and development of functional T FH cells that mediate the B cell responses to T-dependent Ags, it is promising that there is robust Ab reconstitution even in recipients of fully MHC-disparate HSCs by as early as 12 wk post-HSCT. Based on this evidence and previous work suggesting the role of GVHRs in suppressing B cell function (32), we advocate that purified HSC allografts be tested in a clinical context. If these findings translate, HSCT could be used for a wider spectrum of clinical indications, permitting robust immune reconstitution regardless of the genetic disparity between donor and recipient.

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