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*J Immunol* 2007; 178:2893-2900; doi: 10.4049/jimmunol.178.5.2893

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Induced SHIP Deficiency Expands Myeloid Regulatory Cells and Abrogates Graft-versus-Host Disease¹

Kim H. T. Paraiso,* Tomar Ghansah,* Amy Costello,* Robert W. Engelman,† and William G. Kerr‡

Graft-vs-host disease (GVHD)³ is the leading cause of treatment-related mortality in allogeneic bone marrow (BM) transplantation. Immunosuppressive strategies to control GVHD are only partially effective and often lead to life-threatening infections. We previously showed that engraftment of MHC-mismatched BM is enhanced and GVHD abrogated in recipients homozygous for a germline SHIP mutation. In this study, we report the development of a genetic model in which SHIP deficiency can be induced in adult mice. Using this model, we show that the induction of SHIP deficiency in adult mice leads to a rapid and significant expansion of myeloid suppressor cells in peripheral lymphoid tissues. Consistent with expansion of myeloid suppressor cells, splenocytes and lymph node cells from adult mice with induced SHIP deficiency are significantly compromised in their ability to prime allogeneic T cell responses. These results demonstrate that SHIP regulates homeostatic signals for these immunoregulatory cells in adult physiology. Consistent with these findings, induction of SHIP deficiency before receiving a T cell-replete BM graft abrogates acute GVHD. These findings indicate strategies that target SHIP could increase the efficacy and utility of allogeneic BM transplantation, and thereby provide a curative therapy for a wide spectrum of human diseases. The Journal of Immunology, 2007, 178: 2893–2900.

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Received for publication March 29, 2006. Accepted for publication December 21, 2006.

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¹This work was supported in part by grants from the National Institutes of Health (RO1 HL72523) and academic development funds from Moffitt Cancer Center and the University of South Florida. W.G.K. is the Newman Family Scholar of the Leukemia and Lymphoma Society.

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³Abbreviations used in this paper: GVHD, graft-vs-host disease; BM, bone marrow; BMT, BM transplantation; DC, dendritic cell; LN, lymph node; MySC, myeloid suppressor cell; poly(I:C), polyinosinic-polycytidylic acid; WT, wild type.

Copyright © 2007 by The American Association of Immunologists, Inc. 0022-1767/07/$2.00 in mouse embryonic fibroblasts (11). The SHIP locus also encodes a stem cell-specific isoform called s-SHIP that lacks the Src homology 2 domain and is expressed by pluripotent stem cells and tissue-specific stem cells (11, 12). SHIP’s role in signal transduction allows it to regulate cell survival, proliferation, apoptosis, and homeostasis of certain hemopoietic cell types (7, 13–16), as well as primitive stem cell populations (11, 12, 17, 18). Analysis of SHIP-deficient mice revealed that this protein also has a prominent role in the immune system (7, 14, 15, 19). Significant pathologies have been observed in SHIP⁺/⁻ mice, including splenomegaly and an infiltration of myeloid cells into the lungs that contributes to their reduced life span (13, 16, 20).

We previously found that SHIP is critical for maintenance of NK receptor repertoire diversity (15, 21). Disruptions of the NK receptor repertoire caused by germline SHIP deficiency enhance engraftment of BM from donors with complete MHC mismatches (15, 21). Hemopoietic stem cells in SHIP⁺/⁻ mice exhibit spontaneous mobilization and reduced BM retention that may reduce the competitive barrier to engraftment by donor hemopoietic stem cells in SHIP-deficient BMT recipients (17). Furthermore, GVHD is reduced in SHIP⁺/⁻ BMT recipients (15) due to a profound expansion of immunoregulatory MySC in secondary lymphoid tissues (7). Although this confluence of genetic abnormalities may contribute to the reduced viability of germline SHIP⁺/⁻ mice (7, 22), these studies led us to propose that induction of SHIP deficiency for a short period before allogeneic BMT might enhance engraftment and survival even in settings in which donor and host have a complete MHC mismatch (7, 15).

To test the above hypothesis, we developed a genetic model in which SHIP deficiency can be induced in adult recipients using an inducible Cre transgene (23) on a SHIP⁺/⁺ background. We used this model to assess whether induction of SHIP deficiency can trigger an expansion of MySC in adult lymphoid tissues capable of repressing allogeneic T cell responses. In this current study, we find that deletion of SHIP leads to a significant increase in the MySC compartment in both spleen and lymph nodes (LNs) of
adult MxCreSHIPfloxed mice. In addition, splenocytes and LN cells from adult mice rendered SHIP deficient lack the ability to prime allogeneic T cell responses in vitro (24). Consistent with these in vitro findings, mice with induced SHIP deficiency are protected from acute GVHD following a T cell-replete allogeneic BMT. This study provides evidence that SHIP is essential for MYSc homeostasis during normal adult physiology, and suggests that targeting SHIP expression or its activity in adult transplant recipients could be used to modulate the activities of these myeloid regulatory cells for therapeutic purposes.

Materials and Methods

Mice

Mice with germline transmission of a SHIPlox allele were previously created in our laboratory (15) and were maintained by intercrossing SHIPfloxed mice (C57BL/6 background). MxCre transgenic mice were purchased from The Jackson Laboratory. SHIPfloxed and MxCre/SHIPfloxed mice were mated to obtain progeny that are MxCre/SHIPfloxed and SHIPfloxed on a C57BL/6 background. MxCre/SHIPfloxed and SHIPfloxed littermates were generated for the BMT study by intercrossing MxCre/SHIPfloxed and SHIPfloxed mice. All studies were performed in accordance with the guidelines and approval of the Institutional Animal Certification and Use Committee at the University of South Florida.

Conditional deletion of SHIP

MxCre/SHIPfloxed mice were conditionally deleted for SHIP through the i.p. injection of polyinosinic-polycytidylic acid (poly[IC]) (Sigma-Aldrich). SHIPfloxed or SHIPfloxed controls were treated in a similar fashion. Mice were injected three times with 625 μg of poly[IC] on days 1, 4, and 7. For GVHD studies, mice were injected twice with 625 μg of poly[IC] on days 1 and 4 before BMT on day 8. The administration of poly[IC] causes the in vivo production of IFN-α and IFN-β, which activates the Mx1 promoter and Cre recombinase expression (24). Cre recombinase specifically recognizes the loxP sites flanking the promoter and first coding exons of SHIP (15).

PCR confirmation of SHIP deletion

For DNA analysis, mice were bled after poly[IC] injection, and genomic DNA was isolated from PBMC using Qiagen’s DNeasy Kit per the manufacturer’s instructions. The multiplex PCR for SHIP was conducted as previously described (15). The primers for the identification of deleted SHIP alleles are as follows: 3K3 prime, 5′-CCA CAA GTG ATG CTA AGA GAT GC-3′; FRCRE 327-306, 5′-AGT CAC GTC CCA CCA TCC TAT G-3′; 5′-1004, 5′-TCT TCC TGG GCA ATC CTA TG-3′. PCR amplifications produced 3 μl of DNA per reaction. The cycling conditions were step 1: 94°C for 4 min for denaturing; step 2: 94°C for 60 s, 56°C for 50 s, and 72°C for 6 min 30 s; step 2 was repeated 35 times; step 3: 72°C for 10 min. The holding temperature was 4°C. The samples were run on a 3–8% Tris-acetate gel. The gel was then transferred to Hybond-ECL nitrocellulose membrane (Amersham Biosciences). The membrane was blocked with 5% nonfat milk, 1× PBS, and 0.1% Tween 20, and probed with 1 mg/ml P1C1 (primary Ab) and anti-mouse IgG-HRP 1:80,000 (secondary Ab). SHIP protein was detected using Pierce’s Super Signal West Femto chemiluminescent detection reagents. For analysis of SHIP expression on whole cell lysates, 15 μg of protein was loaded per lane.

Flow cytometry analysis

In all experiments, splenocytes and mesenteric LN cells from SHIPfloxed/mice and MxCreSHIPfloxed mice were digested with collagenase D (Roche Molecular Biochemicals) for 90 min at 37°C to increase yield. Collagenase D-processed and RBC-lysed splenocytes and collagenase D-processed mesenteric LN cells were analyzed by flow cytometry with anti-NK1.1, anti-B220, anti-CD3, anti-CD11c, and anti-B7.2 for identification of DC. In addition, MySc were identified using the anti-CD11b (Mac-1) and anti-Gr-1 (Ly6-G). All Abs were purchased from BD Pharmingen.

Mixed leukocyte reaction

Following RBC lysis, SHIPfloxed and MxCreSHIPfloxed splenocytes or LN cells (stimulators) (8 × 10^7/well) were irradiated (2000 rad) and cocultured with BALB/c splenocytes or LN cells (responders) (4 × 10^7/well) in a one-way MLR assay. All cells were plated in triplicate in 96-well U-bottom plates (Costar) containing RPMI 1640 complete medium for 4 days. Cells were pulsed with 1.0 μCi of [3H]thymidine/well for 18 h (post-96 h of MLR assay setup). Cells were lysed and high m.w. DNA captured on glass fiber filters using an automated cell harvester (Packard Instrument). Incorporation of [3H]thymidine was quantitated using a Packard TopCount NXT (Packard Instrument). Specific [3H]thymidine incorporation into genomic DNA was calculated as the average of the mean cpm (±SEM) of triplicate wells. To analyze the suppressive potential of purified myeloid subsets using the one-way MLR assay described above, 3 × 10^6 sorted cells (Mac1+Gr1+), Mac1+Gr1−, or Gr1+Mac1− cells) were added to each MLR well containing 4 × 10^6 stimulators (irradiated wild-type (WT) BL6 splenocytes) and 2 × 10^6 responders (BALB/c splenocytes).

BM1 and GVHD analysis

All SHIPlox and MxCreSHIPlox mice were injected with 625 μg of poly[IC] (i.p.) on days 1 and 4. On day 8, the mice received 950 rad from a 137Cs source as a single dose, and the mice were then transplanted with 15 × 10^6 BM cells and 15 × 10^6 splenocytes from BALB/c (H2d) donors by retro-orbital injection. All recipients were on a C57BL/60 (H2b) background. Mice were maintained on autoclaved bedding, water, and chow in microisolator cages for the duration of the study. The statistical significance of survival differences was assessed by the Kaplan-Meier log rank test with p < 0.05 considered significant. In parallel, we also performed a syngeneic transplant on a cohort of C57BL/6D recipients using 15 × 10^6 whole BM cells and 15 × 10^6 splenocytes from C57BL/6D donors. The clinical manifestations of GVHD were rated on a scale of 0–2 (0, no evidence of disease; 2, clear evidence of disease) and included assessments of weight loss, posture, skin integrity, fur texture, and activity, as described by Cooke et al. (25) Three investigators (K. Paraiso, A. Costello, and W. Kerr) evaluated each mouse at each time point independently of each other, and their scores were averaged.

Results

A novel genetic model to study the impact of induced SHIP deficiency

To assess the impact that induction of SHIP deficiency has on normal adult physiology, we established the MxCreSHIPlox model in which either both SHIP alleles are floxed (MxCreSHIPlox/lox mice) or one allele is floxed with the other having the germline SHIP mutation (MxCreSHIPfloxed/− mice). We initially tested the feasibility of inducing SHIP deficiency in this model by treating two MxCreSHIPlox/lox mice with poly[IC] that activates transcription of the MxCre transgene. Western blot analysis of PBMC obtained immediately before poly[IC] treatment or 10 days later showed that ablation of SHIP expression was achieved in these MxCreSHIPlox/lox mice with SHIP expression being undetectable by Western blot (Fig. 1a). These results indicate induction of SHIP expression is robust in the hemopoietic compartment of MxCreSHIPlox/lox mice following poly[IC] administration. We confirmed deletion of the SHIP WT allele via PCR analysis of genomic DNA (Fig. 1b). For the purpose of discussion in this study, we consider poly[IC]-treated MxCreSHIPlox mice to have full SHIP deficiency if no detectable SHIP allele or protein is present (Fig. 1). However, in some instances, poly[IC]-treated MxCreSHIPlox mice showed a decrease in SHIP protein expression by Western blot, but retained detectable expression of SHIP expression in PBMC (Fig. 1c). We consider these latter mice to have partial SHIP deficiency. To date, our analysis of induced SHIP deficiency in 25 MxCreSHIPlox mice following three
poly(I:C) injections showed that 17 mice exhibited full SHIP deficiency posttreatment, whereas 7 exhibited partial SHIP deficiency, with one mouse showing no significant SHIP deficiency as determined by Western blot analysis of PBMC. There appears to be no correlation with the presence of one floxed allele or two in achieving full SHIP deficiency in this model, and therefore, in our experience either MxCreSHIPflox/flox (Fig. 1d) or MxCreSHIPflox/− can be used interchangeably to induce SHIP deficiency in the adult. With the exception of one mouse who died 10 days after initiation of poly(I:C) treatment, all poly(I:C)-treated MxCreSHIPflox mice survived to at least 21 days following the initiation of SHIP deficiency, at which point they were euthanized for analysis of their hemolymphoid compartment. The analysis of both full and partial SHIP deficiency is germane to the potential development of a clinically applicable SHIP inhibition strategy, and thus, we further analyzed MySC homeostasis and function in mice representative of both groups.

Dendritic cells (DC) are present in normal numbers, but MySC are increased in peripheral lymphoid tissues of adult mice with induced SHIP deficiency

The immune attack by donor T cells that is responsible for GVHD is triggered by host DC in peripheral lymphoid tissues that survive myeloablation (1). In our previous study, we showed that DC are present in normal numbers in the peripheral lymphoid tissues of SHIP−/− mice and that DC sorted from these tissues were capable of priming allogeneic T cells as efficiently as WT APC (7). In the MxCreSHIPflox model, we show that DC are also present in normal

![FIGURE 1](http://www.jimmunol.org/)

**FIGURE 1.** Inducible ablation of SHIP expression in adult MxCreSHIPflox−/+ mice. *a,* Western blot analysis of SHIP in whole cell lysates prepared from PBMCs of MxCreSHIPflox−/+ mice bled before poly(I:C) injection (day 0) and day 10 after poly(I:C) injection. *b,* To confirm deletion of the SHIP WT allele, genomic DNA was prepared from PBMC of a poly(I:C)-treated MxCreSHIPflox−/+ mouse analyzed in *a,* and was analyzed by a multiplex PCR assay that detects WT or null SHIP alleles. Genomic DNA from SHIP−/−, SHIPflox−/−, and SHIPflox−/+ mice was analyzed in parallel as positive and negative controls. *c,* Western blot analysis of SHIP expression in PBMC prepared from MxCreSHIPflox−/+ mice (12–14) or a SHIPflox−/+ mouse that lacks the MxCre transgene (5) following poly(I:C) treatment (day 21). *d,* Western blot analysis of SHIP expression in PBMC prepared from MxCreSHIPflox−/+ mice (2–4) or SHIPflox−/+ mice that lack the MxCre transgene (5–8) following poly(I:C) treatment (day 21).

![FIGURE 2](http://www.jimmunol.org/)

**FIGURE 2.** A normal percentage of DC is found in secondary lymphoid tissues of SHIP-deficient MxCreSHIPflox−/+ mice. *a* and *c,* Flow cytometry analysis of spleens and mesenteric LN from both poly(I:C)-treated (MxCreSHIPflox−/+ or SHIPflox−/+ mice showed no significant difference in the percentage of DC. DC was comprised of cells stained B7.2+ and CD11c+ and lineage-negative panel of Abs (Lin−) that consisted of NK1.1, CD3, and B220 to exclude NK, T, and B cells. *b* and *d,* The mean percentages of DC (B7.2+ CD11c+ Lin−) were calculated for spleen (n = 3) and LN (n = 3) from MxCreSHIPflox−/+ and SHIPflox−/+.
numbers in the spleen (Fig. 2a) and LN (Fig. 2c) following induction of SHIP deficiency. Further statistical analysis of DC numbers in the spleen and LN of MxCreSHIP<sup>flx/−</sup> and control mice showed no significant difference in their representation in these tissues (Fig. 2, b and d), respectively.

We then assessed whether ablation of SHIP expression leads to a significant expansion of the MySC compartment in peripheral lymphoid tissues by analyzing the frequency of MySC in both the spleen and mesenteric LN by flow cytometry. We found a significant expansion of the MySC compartment in both of these peripheral lymphoid tissues (Fig. 3, a and c). The expansion of MySC in MxCreSHIP<sup>flx/−</sup> mice is ~5- to 10-fold in the spleen (Fig. 3b) and ~10- to 20-fold in LN (Fig. 3d) when compared with age-matched SHIP-deficient and control mice treated with poly(I:C). An MxCreSHIP<sup>flx/−</sup> mouse that was observed to have only a partial reduction in SHIP expression following poly(I:C) administration (12 in Fig. 1c) also exhibited a significant expansion of the MySC compartment in both spleen and LN (Fig. 3e). Thus, induction of SHIP deficiency for a short period can expand the MySC compartment and abrogate priming of allogeneic T cell responses, and this can occur even when the hemopoietic compartment is partially SHIP deficient.

**Induction of SHIP deficiency in adult mice compromises priming of allogeneic T cells by peripheral lymphoid tissues**

In our previous studies of germline SHIP deficiency, we found that priming of allogeneic T cell responses, as measured by both proliferation and IL-2 production, was compromised in SHIP<sup>−/−</sup> spleens and LN (7). We proposed then that allogeneic T cell responses were compromised by the expanded numbers of MySC in these tissues (7). To establish definitively that Mac1<sup>+</sup>Gr1<sup>+</sup>MySC are the cell type that mediates suppression of allogeneic T cell responses in these tissues, we sorted Mac1<sup>+</sup>Gr1<sup>+</sup>MySC from SHIP-deficient and WT spleens and tested their ability to suppress a one-way MLR (Fig. 4a). Addition of either SHIP-deficient or WT Mac1<sup>+</sup>Gr1<sup>+</sup>MySC significantly suppressed the MLR (Fig. 4a). However, addition of an equal number of Mac1<sup>+</sup>Gr1<sup>+</sup> cells did not mediate significant suppression in the MLR (p > 0.05). Interestingly, SHIP<sup>−/−</sup> MySC have greater suppressive ability on a per cell basis than WT MySC (p < 0.01) (Fig. 4a), indicating SHIP deficiency may increase both their representation in secondary lymphoid tissues and their suppressive capacity. Because MySC are also expanded in spleen and LN of adult mice following induction of SHIP deficiency, we then examined...
Using a poly(I:C) administration regimen used by Mikkola et al. (26) in SCL^box/box^ mice, we typically find that three consecutive injections of poly(I:C) are sufficient to render most adult MxCreSHIP^box/box^ mice fully SHIP deficient (Fig. 1). However, two poly(I:C) injections typically trigger only partial SHIP deficiency in the overwhelming majority of MxCreSHIP^box/box^ mice. Because full SHIP deficiency might pose a significant threat to viability, we chose to pursue this latter strategy to induce SHIP deficiency. As expected, most MxCreSHIP^box/box^ mice had detectable, but reduced SHIP expression in their PBMC 6 days after the initial poly(I:C) injection, whereas two mice had no detectable SHIP expression (Fig. 5, a–c). The former were considered to be partially SHIP deficient, whereas the latter were considered fully SHIP deficient. Seven days after the induction of SHIP deficiency, we initiated a fully mismatched BMT procedure in both the poly(I:C)-treated MxCreSHIP^box/box^ cohort and the identically treated SHIP^box/box^ cohort. For BMT, the mice were myeloablated by irradiation from a ^137^Cs source (950 cGy) and received 15 × 10^6^ whole BM cells and 15 × 10^6^ spleenocytes from BALB/c (H2d) donors. The MxCreSHIP^box/box^ and SHIP^box/box^ transplant recipients are on a C57BL/6J (H2b) background, and thus are completely mismatched to the donor at all major MHC loci. In parallel, we performed syngeneic BMT on a cohort of C57BL/6J (H2b) mice. Survival was monitored in all three BMT cohorts for 16 wk posttransplant when acute GVHD is manifest. Survival in the syngeneic BMT cohort was 100%, 94% in the SHIP-deficient cohort (MxCreSHIP^box/box^ + poly(I:C)), and 57% in the SHIP-competent cohort (SHIP^box/box^ + poly(I:C)) (Fig. 5d). Comparison of survival in the SHIP-deficient and SHIP-competent allogeneic BMT cohorts by the Kaplan-Meier log rank test indicated there was significant protection from acute and lethal GVHD when SHIP deficiency was induced before transplant (p = 0.040). As expected, survival of the SHIP-competent cohort receiving allogeneic BMT was significantly reduced relative to the syngeneic BMT cohort (p = 0.001). However, the Kaplan-Meier log rank test indicated comparable survival in the SHIP-deficient allogeneic and syngeneic BMT cohorts (p = 0.232) (Fig. 5d). These findings are consistent with our previous allogeneic BMT studies in germline SHIP^-/-^ mice (7, 15), but importantly, they show that induction of SHIP deficiency in the adult just before a fully mismatched, T cell-replete transplant can provide protection from acute GVHD without significant toxicity.

In addition to survival, we also monitored weight and clinical measures of GVHD posttransplant in the SHIP-deficient and SHIP-competent cohorts. Prolonged SHIP deficiency causes wasting in germline SHIP-deficient mice due to a macrophage-mediated consolidation of the lungs (16, 22). However, induction of SHIP deficiency for 1 wk did not cause a significant drop in the weight of the SHIP-deficient cohort relative to the identically treated SHIP-competent cohort (p = 0.39) (Fig. 5e). Despite starting at a comparable weight immediately before BMT, the weight of the SHIP-deficient cohort rebounded and increased significantly relative to that of the SHIP-competent cohort during the acute recovery phase of transplant (p < 0.05) (Fig. 5e). Based on a scoring system that assesses different features of GVHD, including weight loss, skin integrity, fur texture, posture, and activity (25), the SHIP-deficient cohort exhibited fewer or reduced manifestations of GVHD at 3 and 4 wk posttransplant when acute GVHD is typically at its peak (Fig. 5f). Histopathological analysis of GVHD in key target organs (skin, liver, and the gastrointestinal tract) confirmed the presence of GVHD in all mice that succumbed posttransplant (Fig. 6).

FIGURE 4. Induced SHIP deficiency compromises priming of allogeneic T cell responses by cells from secondary lymphoid tissues. a, MySC purified from SHIP-deficient and WT spleens suppress a one-way MLR. [3H]Thymidine uptake by H2d BALB/c responders in the absence of irradiated stimulators (No Stimulators), in the presence of the following: WT (BL6) whole splenocytes (+/+ WS), SHIP^-/-^ (BL6) whole splenocytes (-/- WS), +/+ WS (BL6) plus +/+ MySC (+/+ WS & +/+ MySC), +/+ WS (BL6) plus -/- MySC (+/+ WS & -/- MySC) (+, p < 0.05; **, p < 0.01 by two-tailed Student’s t test). This experiment is representative of two independent MLRs with purified SHIP-deficient MySC. b and c, Splenocytes (b) and mesenteric LN (c) were prepared from mice that showed full deletion, partial deletion, or no deletion of SHIP expression and were used as stimulators for allogeneic (BALB/c) responders in a one-way MLR assay. The bar graphs indicate the total [3H]thymidine uptake 18 h later. These experiments are representative of three independent MLRs with SHIP-deficient mice and control mice. **, p < 0.01; ***, p < 0.001 (by two-tailed Student’s t test).

Whether allogeneic T cell priming by cells from these tissues was also compromised. Indeed, whole splenocytes and LN cells from mice with induced SHIP ablation were found to prime allogeneic T cell responses very poorly relative to cell preparations from similarly treated SHIP^box/-^ mice (Fig. 4, a and c). The MxCreSHIP^box/-^ mice in Fig. 3, a and c, showed expansion of MySC due to full deletion of SHIP expression, whereas the MxCreSHIP^box/-^ mouse from Fig. 3e showed partial ablation of SHIP expression following Cre recombinase induction. Intriguingly, partially ablated mice still exhibit a significant expansion of their MySC compartment in the spleen and LN of these mice (Fig. 3e) and significant repression of allogeneic T cell priming (Fig. 4, a and c). Therefore, induction of SHIP deficiency for relatively short periods can abrogate priming of allogeneic T cell responses in secondary lymphoid tissues even in instances when SHIP expression is not completely ablated.

Induction of SHIP deficiency in adult mice abrogates GVHD following transplant with a T cell-replete, MHC-mismatched BM graft

To test whether induced SHIP deficiency could compromise allogeneic T cell responses in vivo, we established cohorts of MxCreSHIP^box/box^ and SHIP^box/box^ mice for GVHD analysis.
To confirm that mice were engrafted with donor BM, we analyzed multilineage repopulation 8 wk posttransplant in the surviving mice. Flow cytometric analysis of PBMC was used to determine donor BM contribution to the T, B, and myeloid lineages (Fig. 7). This analysis showed that all mice in the SHIP-deficient and SHIP-competent allogeneic BMT cohorts had significant donor repopulation in all three lineages with no significant difference observed between the two cohorts in either T cell or myeloid repopulation (data not shown) or global hemopoietic repopulation.

FIGURE 5. Induction of SHIP deficiency in the host enhances survival in fully MHC-mismatched BMT. a–c, Western blot analysis of SHIP expression in PBMC of representative poly(I:C)-treated SHIP^{flox/flox} (a) and MxCreSHIP^{flox/flox} (b and c) mice before BMT. The mouse in b is representative of the majority of the MxCreSHIP^{flox/flox} cohort that had partial deficiency before transplant, whereas c indicates one of the two animals in the cohort that had no detectable SHIP expression before BMT. All SHIP^{flox/flox} and MxCreSHIP^{flox/flox} mice were injected with 625 μg of poly(I:C) on days 1 and 4. On day 7, mice were bled and PBMC probed for SHIP and β-actin. On day 8, mice received BMT, as described in Materials and Methods. d, Kaplan-Meier step-function of survival in the MxCreSHIP^{flox/flox} (SHIP-deficient) (n = 15) and SHIP^{flox/flox} (SHIP-competent) (n = 14) cohorts following allogeneic BMT. In parallel, we performed syngeneic BMT on a cohort of C57BL/6J recipients, as described in Materials and Methods (p = 0.001, SHIP^{flox/flox} allogeneic BMT vs autologous BMT cohort; p = 0.040, MxCreSHIP^{flox/flox} allogeneic BMT vs SHIP^{flox/flox} allogeneic BMT cohort; p = 0.232, MxCreSHIP^{flox/flox} allogeneic BMT vs autologous BMT cohort). e, Analysis of weight in the SHIP-deficient and SHIP-competent allogeneic BMT cohorts had significant donor repopulation in all three lineages with no significant difference observed between the two cohorts in either T cell or myeloid repopulation (data not shown) or global hemopoietic repopulation.

FIGURE 6. Histopathological evidence of GVHD in mice that succumbed posttransplant. Formalin-fixed tissue sections from all mice that succumbed in the BMT study described above were analyzed for evidence of GVHD in key target organs (skin, liver, and small intestine) in a blinded fashion by a veterinary pathologist (R. Engelmann). All mice that succumbed showed histopathological evidence of GVHD. Examples of observed histopathology referable to GVHD in the skin (a), liver (c), and small intestine (e) are shown, and compared with the skin (b), liver (d), and small intestine (f) of healthy C57BL/6J mice. a, Skin of mice with GVHD showed pyknosis and vacuolation of epidermal cells in the basal layer and a mild lymphocytic infiltrate in the dermis, compared with unaffected skin of a C57BL/6J control (b). Liver of mice with GVHD (c) showed bile duct destruction and regeneration and a moderate, primarily lymphocytic infiltrate in portal areas with attendant destruction of hepatic parenchyma (between arrowheads and within inset), compared with the unaffected liver and portal triads (arrowhead, inset) of a C57BL/6J control (d). Small intestine of mice with GVHD (e) showed glandular destruction, moderate lymphocytic infiltrate, and focal loss of mucosa, compared with the unaffected intestine of a C57BL/6J control (f) (H&E, ×200; insets, ×400).
SHIP-deficient and SHIP-competent allogeneic BMT cohorts. Previous findings that amplification of SHIP-deficient MySC did not influence MySC. The possibility is that SHIP regulates multiple signaling pathways triggered in response to these ligands that promote MySC homeostasis, including Flt3L, G-CSF, and IL-4 (8, 27). It would be intriguing to determine which of these signals SHIP regulates.

Discussion

Our findings provide further evidence that SHIP plays a critical role in the control of MySC homeostasis and function. Analysis of induced SHIP deficiency in the MxCreSHIPflox model demonstrates SHIP controls MySC numbers during normal adult physiology. Consistent with this role, we find evidence of increased MySC function in secondary lymphoid tissues and reduced GVHD following induction of SHIP deficiency in adult mice. Surprisingly, induced SHIP deficiency also enhances B lymphoid reconstitution in allogeneic BMT recipients.

The rapid expansion of the MySC compartment in secondary lymphoid tissues that we observe following induction of SHIP deficiency indicates SHIP is regulating the numbers of these cells in response to normal homeostatic factors that promote their growth, survival, and/or trafficking to secondary lymphoid tissues. It will be intriguing to determine which of these signals SHIP regulates. Several growth factors are potentially involved in peripheral MySC homeostasis, including Flt3L, G-CSF, and IL-4 (8, 27). Consistent with our findings, SHIP is recruited to receptors for all of these ligands (28–31), and thus may oppose PI3K effector pathways triggered in response to these ligands that promote MySC survival and/or proliferation in secondary lymphoid tissues. Because SHIP can also limit chemotaxis in response to chemokines (32), it is also possible that SHIP regulates trafficking of MySC precursors into secondary lymphoid tissues. A distinct possibility is that SHIP regulates multiple signaling pathways in MySC.

In addition to regulating MySC numbers, SHIP deficiency may also regulate their effector functions. This is suggested by our previous findings that amplification of SHIP-deficient MySC did not compromise the ability of APC in spleen and LN to prime naive, Ag-specific CD4 or CD8 T cell responses (7). Others have shown that Mac1+Gr1+ MySC present in spleens of tumor-bearing mice antagonize APC priming of Ag-specific CD8 T cell responses (33). These disparate findings suggest the possibility that SHIP-deficient MySC are qualitatively different from their WT counterparts. Consistent with this possibility, we find in the current study that purified SHIP-deficient MySC are more suppressive of allogeneic T cell responses than their WT counterparts on a per cell basis. The molecular mechanisms that underlie the functional differences between SHIP-deficient and SHIP-competent MySC merit further investigation, because they could have important implications for tumor immunotherapy and allogeneic BMT protocols to treat malignancy in which graft-vs-tumor responses by T cells play an important therapeutic role. Alternatively, the SHIP-deficient Mac1+Gr1+ cells described in this work and previously (7) could represent a MySC subset with distinct functional properties preferentially amplified by SHIP deficiency in secondary lymphoid tissues.

A potentially important, but unanticipated finding of this study is the small, but significant increase in donor B lymphoid repopulation that we observe in SHIP-deficient allogeneic BMT recipients. As shown by Good and colleagues (34), humoral immune responses to T-dependent Ags are severely compromised following allogeneic BMT. Consistent with this seminal study in a murine allogeneic BMT model, recovery of B cell function and Ab responses to pathogens is frequently delayed or permanently compromised in clinical allogeneic BMT patients (35–38) and contributes to posttransplant morbidity (39). We propose that the enhanced donor B cell repopulation observed in SHIP-deficient allogeneic BMT recipients may also contribute to the improved survival we observe in these recipients. The mechanism responsible for enhanced donor B cell repopulation in SHIP-deficient hosts remains to be defined. However, we note that residual host B lymphopoiesis was shown recently to limit donor B cell repopulation in an allogeneic murine BMT model (40), and that B lymphopoiesis is partially compromised by SHIP deficiency (22).

Our findings lend credence to the possibility of using reversible SHIP inhibition strategies to abrogate GVHD in allogeneic BMT and potentially also host T cell responses that mediate graft rejection in solid organ transplantation. Although therapeutic agents currently exist to control such deleterious T cell activities, these have the drawback of being broadly inhibitory for T cell function, and thus place the transplant recipient at risk for opportunistic infections, tumor relapse, and secondary malignancies (41–43). Our studies and those of others indicate SHIP deficiency does not significantly impair Ag-specific T cell priming (7) or humoral responses to complex Ags (14, 44). Thus, pursuit of reversible SHIP inhibition strategies could potentially provide a more selective form of immune suppression better suited for allogeneic transplant regimens. Although full and prolonged SHIP deficiency clearly has deleterious consequences (22), our findings indicate transplant success can be achieved with induced SHIP deficiency even when a state of partial SHIP deficiency exists.

Most patients who could potentially benefit from an allogeneic BMT procedure are unable to find a donor with an appropriate HLA match. They must then forego a potentially efficacious therapy due to the heightened risk of GVHD from an unmatched donor. However, in murine allogeneic BMT recipients with induced SHIP deficiency, we find they are protected from lethal acute GVHD despite a complete MHC mismatch with the donor. If this finding can be extrapolated to the humans, then clinical BMT procedures involving a significant degree of HLA incompatibility might be feasible. The application of induced SHIP deficiency in this manner would profoundly increase the utility of this curative therapy in a wide spectrum of human maladies, including genetic, autoimmune, and malignant disease.

Acknowledgments

We acknowledge the H. Lee Moffitt Flow Cytometry Facility, Davina Ramos for genotyping of mice, and Amy L. Hazen for confirming the status of SHIP protein expression in mice.
Disclosures

The authors have no financial conflict of interest.

References


