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Expansion of donor-reactive host T cells in primary graft failure after allogeneic hematopoietic SCT following reduced-intensity conditioning

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Running title: Expansion of host T cells in primary graft failure

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Abstract

Graft rejection remains a major obstacle in allogeneic hematopoietic stem cell transplantation following reduced-intensity conditioning (RIC-SCT), particularly after cord blood transplantation (CBT). In a murine MHC-mismatched model of RIC-SCT, primary graft rejection was associated with activation and expansion of donor-reactive host T cells in peripheral blood and bone marrow early after SCT. Donor-derived dendritic cells are at least partly involved in host T cell activation. We then evaluated if such an expansion of host T cells could be associated with graft rejection after RIC-CBT. Expansion of residual host lymphocytes was observed in 4 / 7 patients with graft rejection at three weeks after CBT, but in none of the 17 patients who achieved engraftment. These results suggest the crucial role of residual host T cells after RIC-SCT in graft rejection and expansion of host T cells could be a marker of graft rejection. Development of more efficient T cell-suppressive conditioning regimens may be necessary in the context of RIC-SCT.

Keywords: graft rejection, bone marrow transplantation, cord blood transplantation

Introduction

Allogeneic hematopoietic SCT is a curative therapy for various hematologic malignant tumors, bone marrow failure, and congenital immune and metabolic disorders. The success of SCT is highly dependent on the suppression of recipient immune system to prevent graft rejection by host immunocompetent cells. Progress on SCT has minimized the rate of graft rejection by the selection of HLA-matched donor, the use of myeloablative conditioning regimens, and infusion of large amount of hematopoietic stem cells (HSCs) ¹⁻⁶. However, the incidence of graft rejection is again increasing as the wider application of allogeneic SCT with the increasing use of HLA-mismatched donors, reduced-intensity conditioning (RIC) which could retain host immune cells and HSCs compared to myeloablative conditioning, and cord blood that contains small amount of HSCs compared to G-CSF-mobilized peripheral blood or bone marrow ⁷⁻⁹.

Graft failure or graft rejection has been defined as either lack of initial engraftment of donor cells, or loss of donor cells after initial engraftment ¹⁰. Rejection is a major cause of graft failure and caused by recipient T cells, natural killer (NK) cells, or antibodies ¹⁰⁻¹⁵. An increase in number of residual host T cells is associated with graft

rejection in pediatric allogeneic transplantation¹⁶. HLA-C mismatch with NK epitope mismatching in the rejection direction are associated with higher rates of graft rejection after HLA-mismatched transplantation^{17,18}. Positive serum crossmatch is predictive for graft failure in HLA-mismatched allogeneic SCT^{19,20}.

Thus, fate of transplanted donor HSCs, namely engraftment or rejection is determined by the competition between donor-derived and residual host-derived HSCs (stem cell competition) and by the competition between donor-derived and host-derived immune competent cells, such as T cells, NK cells and B cells (immunological competition)¹¹⁻¹³.

We herein investigated the process of host T cell-mediated immunological graft rejection in a mouse model of RIC-SCT using sublethal irradiation conditioning²¹ and in patients who underwent cord blood transplantation following RIC (RIC-CBT) that represents higher risk for graft rejection than other types of allogeneic SCT.

Materials and Methods

A mouse model of BMT Female C57BL/6 (B6: H-2^b, CD45.2⁺), B6D2F1 (H-2^{b/d}, CD45.2⁺), DBA/2 (H-2^d, CD45.2⁺), B6-Ly5a (H-2^b, CD45.1⁺), and B6.FVB-Tg (Itgax-DTR/EGFP) 57Lan/J (B6.CD11c-DTR, H-2^b) mice were purchased from Charles River Japan (Yokohama, Japan), the Jackson Laboratories (Bar Harbor, ME), and the Animal Resources Centre (Perth, Western Australia, Australia). B6.CD11c-DTR × DBA/2 F1 mice were generated by breeding B6.CD11c-DTR mice with DBA/2 mice. All experiments involving animals were performed under the auspices of the institutional animal ethics committee.

Following 6 or 10 Gy TBI (X-ray), B6 mice were injected with 5×10^6 NK cell-depleted BM cells from allogeneic B6D2F1 or syngeneic B6-Ly5a donor mice on day 0. NK cell depletion of BM cells was performed using CD49b (DX5) microbeads and the autoMACS (Miltenyi Biotec Japan, Tokyo, Japan) according to the manufacturer's instructions. Mice were housed in sterilized microisolator cages and received autoclaved hyperchlorinated drinking water for the first three weeks after BMT.

For donor DC depletion, all recipients were injected with 100 ng diphtheria toxin (DT) intraperitoneally every other day from day 0 until the end of experiments.

After transplant, complete blood counts were performed using a Celltac MEK-6358 (Nihon Kohden, Tokyo, Japan). Donor cell chimerism was evaluated weekly in the peripheral blood. Monoclonal antibodies (mAbs) used were CD4, CD8, CD45.1, CD45.2, TCR β , H-2K^b, and H-2K^d (BD Biosciences, San Diego, CA). Donor and host cells in a BDF1 \rightarrow B6 BMT were identified as H-2K^{b+} H-2K^{d+} and H-2K^{b+} H-2K^{d-}, respectively. In a B6-Ly5a \rightarrow B6 syngeneic BMT, CD45.1 and CD45.2 were used as donor and host cell specific markers, respectively. Complete donor cell engraftment, mixed donor and host chimerism, and rejection were defined as >95%, 5-95%, and <5% donor chimerism of WBCs in peripheral blood, respectively. For intracellular IFN- γ staining, cells were incubated for 4 h with Leukocyte Activation Cocktail (BD Biosciences) at 37°C. Then, the cells underwent permeabilization with BD Cytofix/cytoperm solution (BD Bioscience) and were stained with FITC-conjugated IFN- γ mAbs (BD Bioscience). Dead cells were identified as 7-Amino-actinomycin D (7-AAD; BD Biosciences)-positive cells. The cells were analyzed using a FACS Calibur

flow cytometer (BD Biosciences) and data was analyzed using a Flow Jo software (Tree Star, San Carlos, CA).

CTL assays Fourteen days after BMT, T cells were separated from BM of the bilateral tibias and femurs or spleen using Thy1.2 (CD90.2) microbeads and the autoMACS according to the manufacturer's instructions. The percentage of CD8⁺ cells in this fraction was determined by flow cytometry and counts were normalized for CD8⁺ cell numbers. CTL assays were performed as previously described²². Briefly, tumor targets, 2×10^6 DBA/2-derived mastocytoma cell line P815 (H-2^d) or B6-derived lymphoma cell line EL4 (H-2^b), were labeled with 100 μ Ci of ⁵¹Cr sodium salt (Amersham Biosciences, Tokyo, Japan) for 2 h. After washing three times, the labeled targets were plated at 10^4 cells per well in U-bottom plates (Corning-Costar Corp., Cambridge, MA). Allogeneic T cell preparations, as described above, were added to quadruplicate wells at varying effector-to-target ratios and incubated for 4 h. Maximal and background release were determined by adding 1% SDS or media alone to the targets, respectively. ⁵¹Cr activity in the supernatants collected 4 h later were determined using a Wallac 1470 WIZARD[®] Gamma Counter (Wallac Oy, Turku,

Finland), and lysis was expressed as a percentage of maximum: percentage of specific

lysis = $100 \times (\text{sample count} - \text{background count}) / (\text{maximum count} - \text{background count})$.

For CTL assay against hematopoietic cells, CD8⁺ cells were separated from BM of the

bilateral tibias and femurs using CD8 microbeads and the midiMACS 21 days after

BMT. Donor and host-derived hematopoietic target cells were isolated from BM of

naïve B6-Ly5a and B6D2F1 mice by depleting lineage committed cells using

biotin-conjugated CD3, CD5, Ter119, Gr1, Mac1, B220 antibodies and BD IMagTM

Streptavidin Particles Plus-DM (BD Bioscience). The lineage negative targets were

plated at 3×10^3 cells per well in 96 U-bottom plates with 1.5×10^5 CD8⁺ cells and

incubated for 4 hours. After incubation the cells were collected and stained with target

marker (H2Dd or CD45.1), lineage, Sca-1, c-kit, Annexin V and SYTOX blue stain

(Invitrogen) for FACS analysis (Fortessa LSD, BD Bioscience)²³.

Patients We retrospectively analyzed data from 24 adult patients who underwent

RIC-CBT at Kyushu University Hospital, Hamanomachi Hospital and Kitakyushu

Medical Center between August 2003 and February 2007 and survived more than 28

days posttransplant. Patients who received the second CBT for rejection after primary transplant were excluded. This study was approved by the institutional review board of each participating institute.

Transplantation procedure and definitions Cord blood units were obtained from the Japan Cord Blood Bank Network. Serological typing for HLA-A, -B, and -DR antigens of cord blood units and patients' blood samples was performed. HLA-mismatch in the graft-versus-host (GVH) vector was defined when the recipient's antigens were not shared by the donor, while mismatch in the host-versus-graft (HVG) vector was defined as when the donor's antigens were not shared by the recipient. RIC regimens were defined as previously reported²⁴⁻²⁶. Regimens used were fludarabine 150-180 mg/m² with either cyclophosphamide 60mg/kg, busulfan 8mg/kg, or melphalan 80-140 mg/m² with 4 Gy TBI. The prophylaxis regimens for GVHD were cyclosporine (CSP) or tacrolimus alone, CSP plus methotrexate (MTX), or CSP plus mycophenolate mofetil (MMF). Risk status at transplantation was categorized as either standard-risk or high-risk. Standard-risk diseases included acute leukemia in first complete remission,

chronic myelogenous leukemia in first chronic phase, and refractory anemia of myelodysplastic syndrome. Other diseases were categorized as high-risk disease. Graft failure was defined as failure of the absolute neutrophil count to surpass 0.5×10^9 /L for 3 consecutive days before relapse, second transplantation, or death ²⁷. Chimerism of WBCs was assessed 3 weeks after CBT by using fluorescence in situ hybridization in sex-mismatched donor-recipient pairs or polymerase chain reaction (PCR) for variable numbers of tandem repeats in sex-matched pairs at a sensitivity of 10% ²⁸.

Statistical analysis Mann-Whitney U tests were used to analyze cell counts and clinical scores. We defined $P < 0.05$ as statistically significant. The values are described with standard error (SE).

Results

Intensity of TBI impacts donor cell engraftment We first examined the impact of conditioning intensity on donor cell engraftment in a murine MHC-mismatched B6D2F1 (H-2^{b/d}) → B6 (H-2^b) model (F1 → P) of BMT ²¹. In this model, donor T cells are tolerant of the recipient MHC, but donor NK cells not expressing H-2^b-specific Ly49C/I inhibitory receptors and bearing instead H-2^d-specific Ly49A/G2 receptors can be activated to kill the recipient's targets ²⁹. In contrast, recipient T cells are activated by the donor-derived alloantigens, whereas recipient NK cells are not reactive to the donor's targets. Therefore, graft rejection is primarily mediated by donor-reactive host T cells in this model. Donor NK cells that can compete with host T cells were depleted from the donor inoculum. B6 mice were exposed to 6 or 10 Gy TBI and then intravenously injected with 5×10^6 NK cell-depleted BM cells from B6D2F1 mice or B6-Ly5a mice on day 0. It has been shown that conditioning with ≤ 7 Gy TBI is not lethal in this strain combination ²¹. After BMT, donor cell chimerism was evaluated in the peripheral blood weekly. WBC counts recovered rapidly to the normal level by 4 weeks after syngeneic BMT and allogeneic BMT following 10 Gy TBI, but not after

allogeneic BMT following 6 Gy TBI (Figure 1A). Complete donor chimerism was rapidly achieved after allogeneic BMT following 10 Gy TBI, whereas donor chimerism never exceeded 10 % after allogeneic BMT following 6 Gy TBI (Figure 1B). In contrast, graft failure did not develop in the syngeneic recipients with 6 Gy TBI, confirming that the graft rejection in the allogeneic recipients with 6 Gy TBI resulted from alloreactivity but not insufficient amount of stem cells.

Temporal expansion of host T cells in the peripheral blood in mice experiencing graft

rejection We enumerated donor and host T cells separately after BMT. The number of donor T cells (H-2K^{b+} H-2K^{d+} TCRβ⁺) in peripheral blood increased rapidly in the allogeneic recipients with 10 Gy TBI and the syngeneic recipients with 6 Gy TBI but not in the allogeneic recipients with 6 Gy TBI (Figure 1C). Such an increase of donor T cells in these animals is likely mediated by homeostatic proliferation in lymphopenia after BMT. Residual host T cells (H-2K^{b+} H-2K^{d-} TCRβ⁺) accounted for only 10 % of total T cells at 6 weeks after BMT in the allogeneic recipients with 10 Gy TBI (Figure 1D). In contrast, host T cells markedly increased with a steep peak at 3 weeks after

BMT in the allogeneic recipients with 6 Gy TBI. Such a surge of residual host T cells which consisted of 99 % of total T cells was not seen in either of the allogeneic recipients with 10 Gy TBI or the syngeneic recipients with 6 Gy TBI. Given that those graft rejections were not caused by insufficient number of stem cells (Figure 1B), we hypothesized that the expansion of host T cells in the allogeneic recipients with 6 Gy TBI could be responsible for primary graft rejection.

Host T cell expansion is associated with early rejection of donor BM graft We

examined whether the activated host T cells could expand in not only peripheral blood but also hematopoietic organs where donor graft cells migrate. B6 recipients irradiated with 6 Gy TBI were intravenously injected with 5×10^6 NK cell-depleted BM cells from allogeneic B6D2F1 mice or syngeneic B6-Ly5a mice. Two weeks later when donor chimerism has already been lost in allogeneic recipients (Figure 1B), cells were isolated from BM and spleen to analyze chimerism and proliferation of host T cells. In allogeneic animals, host-derived cells (H2-K^{b+} H-2K^{d-}) exceeded 99 % in BM (99.6% ± 0.34 %) and spleen (99.6 % ± 0.30 %), whereas it was 44.7 % ± 22.1 % in BM and

48.4 % \pm 32.2 % in spleen of syngeneic recipients (data not shown). Host-derived IFN- γ^+ CD8 $^+$ T cells markedly expanded in the BM and spleen from allogeneic recipients compared to those in syngeneic recipients both at 2 weeks (Figure 2A) and 3 weeks after BMT (Figure 2B). In contrast, host-derived IFN- γ^+ CD4 $^+$ T cells were modestly increased in spleen 2 weeks after BMT and in BM 3 weeks after BMT in allogeneic recipients. Frequencies of host-derived IFN- γ^+ T cells were also increased in BM (Figure 2C). We next evaluated the cytolytic activity of those host CD8 $^+$ T cells with respect to donor targets. BM T cells were isolated from allogeneic recipients treated with 6 Gy TBI two weeks after BMT. We confirmed with a flow cytometric analysis that more than 99% of these T cells were host-derived (data not shown). These T cells exhibited CTL activity against donor-type P815 (H-2 d) targets but not against donor-type EL4 (H-2 b) targets (Figure 2D). Similar results were obtained when splenic T cells were used (data not shown). Furthermore, the CD8 $^+$ T cells isolated from BM of graft-rejected recipients targeted and induced cell death of donor-type HSCs (lineage $^-$ c-Kit $^+$ Sca-1 $^+$ cells) but not host-type HSCs as Annexin V $^+$ SYTOX $^+$ cells (Figure 2E). These results suggest that the expansion of donor-reactive host T cells in hematopoietic

tissue including BM precedes the transient increase of host T cell in peripheral blood in the allogeneic recipients who experienced primary graft rejection.

Donor dendritic cell depletion abolished the transient host T cell expansion and

improved donor chimerism partially Next we examined if donor cell rejection could

be prevented when host T-cell activation and expansion were inhibited. Donor DCs

were expected as the main population to activate host T cells³⁰. B6 recipients irradiated

with 6 Gy TBI were transplanted with 5×10^6 NK cell-depleted BM cells from

allogeneic wild-type B6D2F1 or B6.CD11c-DTR \times DBA/2F1 mice. All recipients were

injected with 100 ng DT intraperitoneally every other day from day 0. We evaluated

kinetics of host T-cell expansion and donor chimerism in peripheral blood every week.

Host T-cell expansion was suppressed (Figure 3A) and the donor chimerism at three

weeks after BMT was increased in donor DC-depleted recipients compared to

DC-replete controls (Figure 3B).

Host T cell surge in the patients who resulted in primary graft rejection following

RI-CBT

We next investigated clinical relevance of such a “surge” of host T cells in rejection in adult patients who underwent RIC-CBT retrospectively. We focused on RIC-CBT, because primary graft rejection was rare after BMT or peripheral blood stem cell transplantation ⁹. In 24 adult patients who underwent RIC-CBT, 17 patients achieved engraftment at a median of 22 days (range, 13-31 days), while primary graft rejection occurred in 7 patients (Table 1). The number of infused cells, CD34⁺ cells and HLA disparity were evenly distributed between the groups (Table 1). We clearly observed a surge of lymphocytes in 4 out of 7 patients who experienced primary graft rejection (Figure 4A), but not in patients with engraftment (Figure 4B). The chimerism analysis at 3 weeks after CBT demonstrated that expanded lymphocytes were host-derived (Figure 4C). One out of 4 patients with the transient lymphocyte increase had aplastic anemia as the original disease and the other three patients received single course of cytotoxic chemotherapy for the original malignant diseases prior to CBT. A flow cytometric analysis of these host lymphocytes was performed in one representative patient with rejection; 85% of the cells were CD8⁺ T cells (data not shown).

Discussion

Graft rejection is mediated by infusion of insufficient numbers of HSCs and/or humoral and cellular immunological mechanisms, involving anti-HLA antibodies and donor-reactive host T cells and NK cells^{10, 31}. Although standard myeloablative conditioning is usually sufficient to suppress donor-reactive host T cells and permits donor cell engraftment, RIC leaves larger numbers of residual host immunocompetent cells than myeloablative conditioning. However, the impacts of RIC on the kinetics of host T cells in the context of graft rejection have not been well delineated.

We first evaluated effects of nonmyeloablative conditioning on host T cells in a mouse F1 → P model with donor NK cell depletion, where recipient T cells can recognize donor alloantigens, while donor T cells cannot recognize recipient alloantigens. This model thus represents a simplified model that allows us to examine the effects of conditioning intensity on the ability of anti-donor host T cells to mediate graft rejection independent of the presence of donor-derived T cells and NK cells, although mechanisms of clinical rejection are more complex, involving donor T and NK cell responses. In this model, the conditioning with 6 Gy TBI resulted in primary graft

rejection, whereas syngeneic BMT with the same number of BM cells following 6 Gy TBI achieved donor dominant chimerism, indicating the T-cell mediated graft rejection, not due to insufficient number of stem cells in graft. The conditioning with 10 Gy TBI resulted in donor dominant chimerism (> 98 % donor cells in WBC) 6 weeks after BMT, although there are some residual host-derived T cells. However, these residual T cells did not induce graft rejection probably due to their impaired function by 10 Gy TBI.

We found graft rejection was associated with an increase in numbers of host T cells in peripheral blood at three weeks after BMT and expansion of cytotoxic CD8⁺ T cells in the hematopoietic tissues, BM and spleen. These results corresponded with the previous experimental studies that CD8-deficient recipient mice were superior for engraftment^{32, 33} and that preconditioning with anti-CD8 mAb enhanced allogeneic engraftment³⁴.

Given that host T cells selectively targeted donor-type cells (Figure 2), these host T cells should recognize donor-type MHC and minor histocompatibility antigens directly presented on donor APCs or indirectly presented on host APCs. Although the dominant pathway of allorecognition by host T cells is difficult to be discriminated in

our model, these findings lead us to the hypothesis that the inhibition of donor alloantigen presentation to host T cells could permit donor cell engraftment^{30,35}. Donor DC depletion by DT administration suppressed the transient host T cell expansion and delayed graft rejection with transient mixed chimerism, but failed to maintain sustained engraftment. This is likely due to the presence of other subsets of donor-derived APCs than CD11c⁺ DCs, as well as insufficient DC depletion after multiple injections of DT as has been reported³⁶. Nonetheless, our results are consistent with a previous study showing that donor APCs, not host APCs, particularly DCs play a critical role in eliciting bone marrow rejection³⁰. However, evidence suggesting the causative role of donor DCs in BM rejection is still limited and therefore there is also the possibility that the other components of donor or host cells play a role in inducing rejection^{35, 37}. Nonetheless, our results suggest that primary graft rejection is at least partly mediated by host T-cells stimulated by donor DCs.

We then evaluated relevance of the overshoot of host T cells observed after MHC-mismatched RIC-SCT in a mouse model in patients experiencing graft rejection after HLA-mismatched RIC-CBT, since rejection is more frequent after CBT³⁸. Four

out of the seven patients experienced graft rejection had the temporal increase in numbers of host-derived lymphocytes at 3 weeks after RIC-CBT, similar to the observation in the murine model. We confirmed that more than 80% of these lymphocytes are CD8⁺ T cells in one patient. Our results are consistent with previous several clinical studies demonstrating the presence of host-derived, anti-donor T cells in recipients experienced graft rejection after CBT³⁹⁻⁴³. In addition to lower numbers of HSCs infused in CBT, lower numbers and impaired function of CB T cells and/or APCs may be associated with host T cell expansion and graft rejection after CBT⁴⁴⁻⁴⁸. Further studies are also required to evaluate naïve versus memory phenotype of host T cells expanded in graft rejection to determine a role of host memory T cells which had been sensitized by prior transfusion.

In conclusion, our results suggest a crucial role of host-derived anti-donor T cells in primary graft rejection after RIC-SCT and lead us to speculate that developing conditionings to suppress host T cells efficiently is urgently required for RIC-CBT.

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MK, DH, KA, YS performed the research, MK, KN, TE, YO, TM analysed patient data,

MK and TT designed the research study and wrote the paper.

Footnotes

Abbreviations used in this paper: CBT, cord blood transplantation; DT, diphtheria toxin; HSC, hematopoietic stem cell; RIC, reduced-intensity conditioning.

Conflict of interest: the authors have no competing interests.

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Figure Legends

Figure 1. The impact of TBI doses on donor cell engraftment

B6 mice were exposed to 10Gy TBI (n=6, closed circle with solid line) or 6Gy TBI (n=9; closed triangle with solid line), and intravenously injected with 5×10^6 NK cell-depleted BM cells from B6D2F1 mice on day 0. In syngeneic controls, B6 mice were transplanted with cells from B6-Ly5a mice following 6Gy TBI (n=6, open circle with broken line). Numbers (A) and donor chimerism (B) of WBCs after BMT.

Numbers of donor (C) and host (D) T cells. Donor T cells were H2-K^{b+} H-2K^{d+} TCR β ⁺ cells and host T cells were H2-K^{b+} H-2K^{d-} TCR β ⁺ cells. Results from three similar experiments were combined (N=6-9 per group). * $p < 0.01$ compared with 2 w and 4 w.

Data are shown as the mean \pm SE.

Figure 2. Expansion of anti-donor cytotoxic host T cells

B6 mice received 6Gy TBI and then injected intravenously with 5×10^6 NK cell-depleted BM cells from B6D2F1 (allo) or B6-Ly5a mice (syn). Splenocytes and BM cells were isolated from the bilateral tibias and femurs 2 and 3 weeks after BMT.

(A, B) The absolute numbers of IFN γ ⁺ CD4⁺ cells (gray bar) and IFN γ ⁺ CD8⁺ cells

(black bar) in the BM and spleen 2 weeks **(A)** and 3 weeks after BMT **(B)**. **(C)** Frequencies of IFN γ ⁺ CD4⁺ cells (gray bar) and IFN γ ⁺ CD8⁺ cells (black bar) in the BM and spleen 3 weeks after BMT. **(D, E)** BM T cells were cultured with P815 (H-2^d) targets or EL4 (H-2^b) targets at varying effector-to-target ratios **(D)**, or with lineage negative BM cells from naïve donor-type B6D2F1 (H-2^{b/d}) mouse or host-type B6-Ly5a mice (CD45.1⁺) **(E)** to determine cell cytotoxicity. Data shown are representative of two replicate experiments and mean \pm SE ($n = 4-10$ per group). *P <0.01, **P <0.0001 compared with syn.

Figure 3. Donor DC depletion improves donor chimerism while abolishing the transient host T cell increase

B6 recipients irradiated with 6 Gy were transplanted with 5×10^6 NK cell-depleted BM cells from allogeneic wild-type B6D2F1 or B6.CD11c-DTR \times DBA/2F1 mice. All recipients were injected with 100 ng DT intraperitoneally every other day from day 0. Time-course of the numbers of host T cells **(A)** and chimerism at 3 weeks after BMT **(B)** in peripheral blood are shown. Data are shown as the mean \pm SE ($n = 5$ per group).

Figure 4. A surge of host lymphocytes in patients with graft rejection after

RI-CBT

Numbers of lymphocytes in peripheral blood of patients with rejection (**A**) and those without rejection (**B**) after RI-CBT. (**C**) Numbers of host-derived lymphocytes 3 weeks posttransplant were the products of numbers of lymphocytes and percentage of host-derived cells in peripheral blood.

Table 1

Variable	Graft rejection	Engraftment
No. of patients	7	17
Age (years), median (range)	63 (29-65)	40 (23-65)
Risk status (standard risk / high risk)	4 / 3	4 / 13
Primary diseases		
Hematological malignancies	6	16
Aplastic anemia	1	1
No. of infused nuclear cells, median (range), x10 ⁷ /kg	3.06 (2.05-3.20)	2.94 (1.69-6.02)
No. of infused CD34+ cells, median (range), x10 ⁵ /kg	0.87 (0.42-1.43)	1.00 (0.70-1.83)
HLA-mismatch in the graft-versus-host vector		
0	0	0
1	4	4
2	3	9
More than 3	0	4
GVHD prophylaxis		
CSP or FK506 alone	0	5
CSP + sMTX	6	6
CSP + MMF	1	6
Day of engraftment, median (range)	NA	22 (13-31)

CR, complete remission; Flu, fludarabine; BU, busulphan; CY, cyclophosphamide; LPAM, Melphalan; CL, cladribine; sMTX, short term methotrexate; CSP, cyclosporine; MMF, mycophenolate mofetil; NA, not applicable

Figure 1

Figure 1

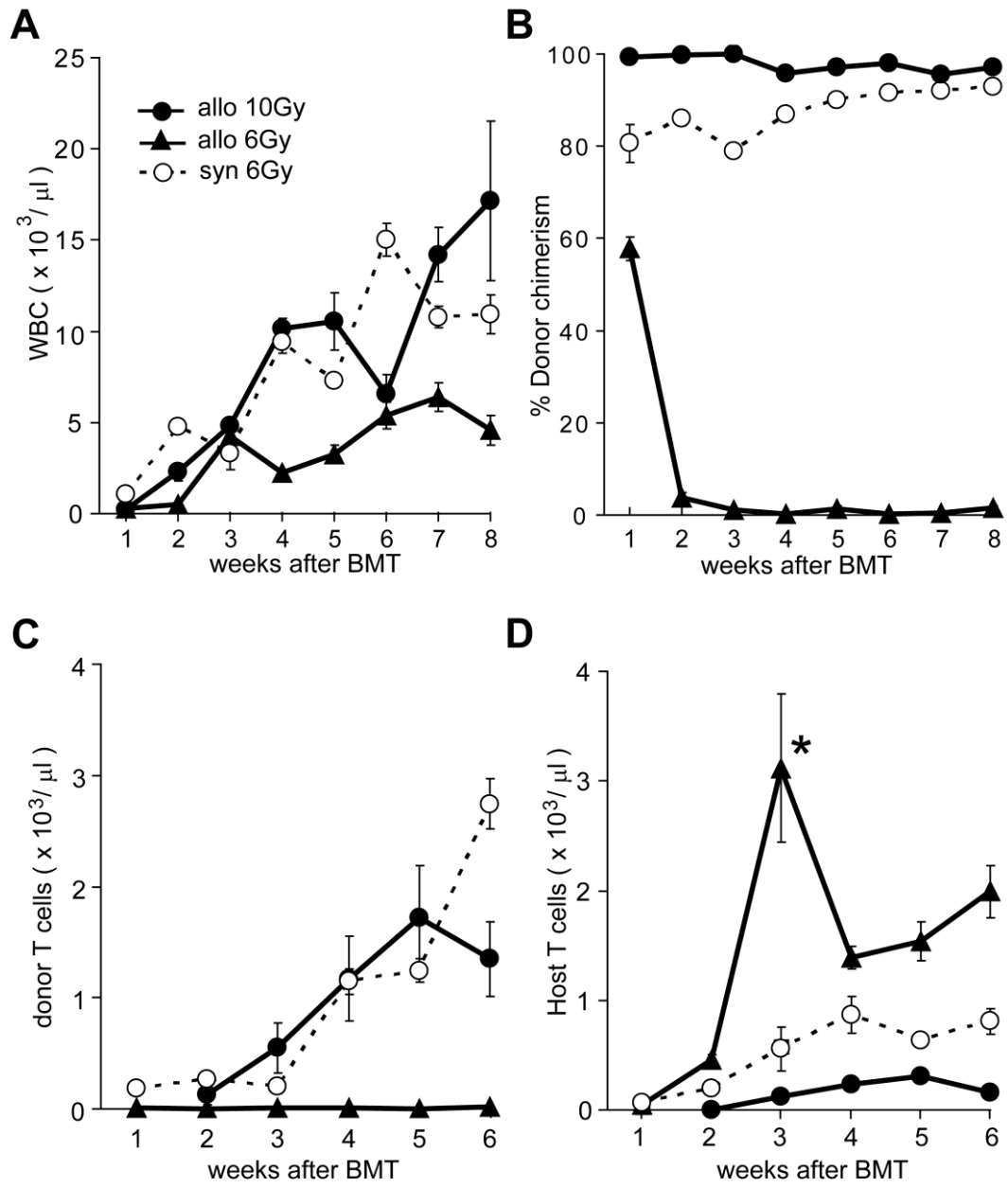


Figure 2

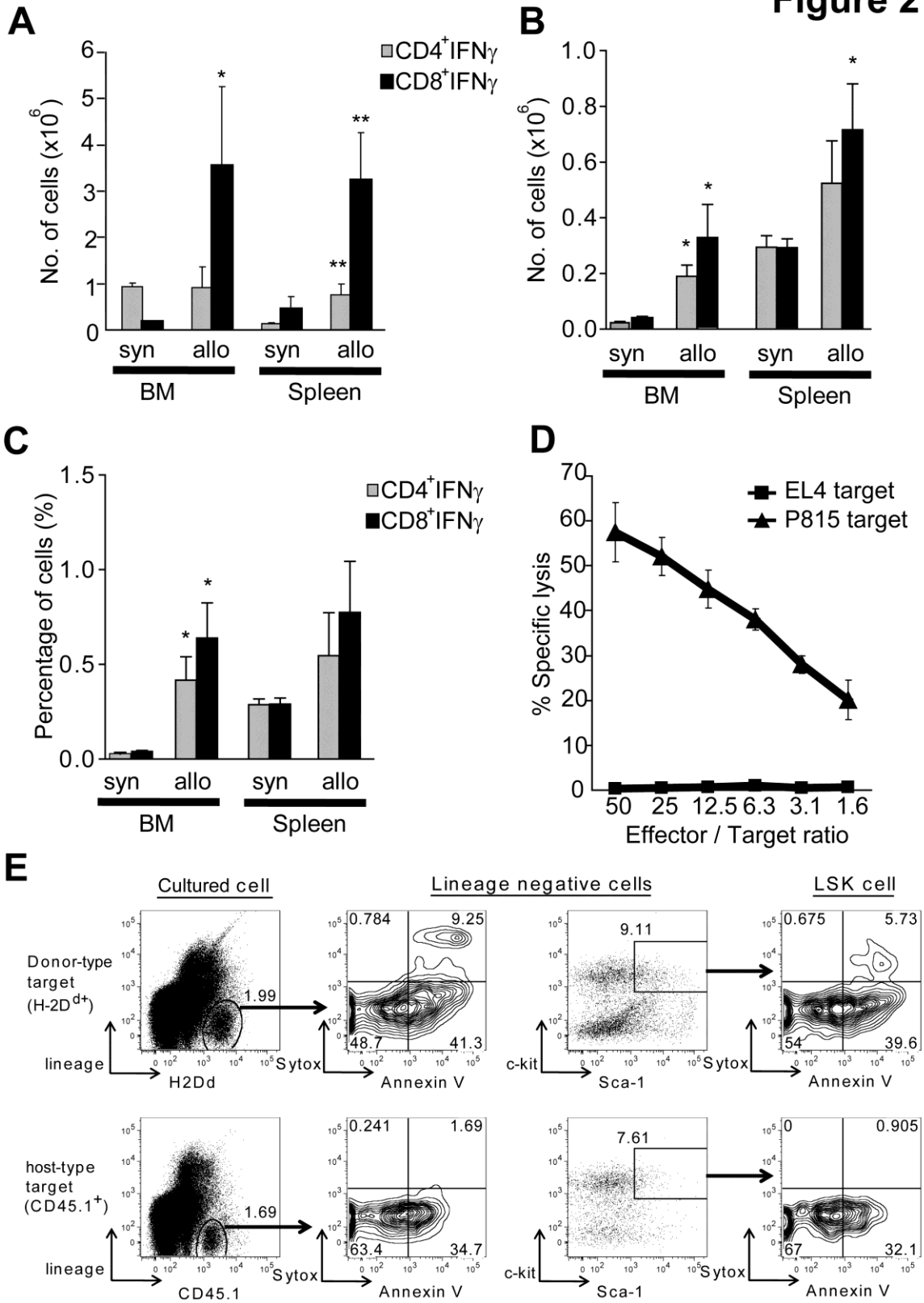
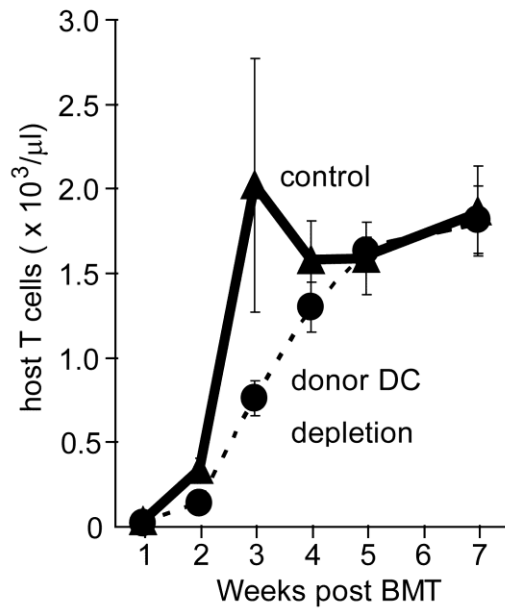


Figure 3

A



B

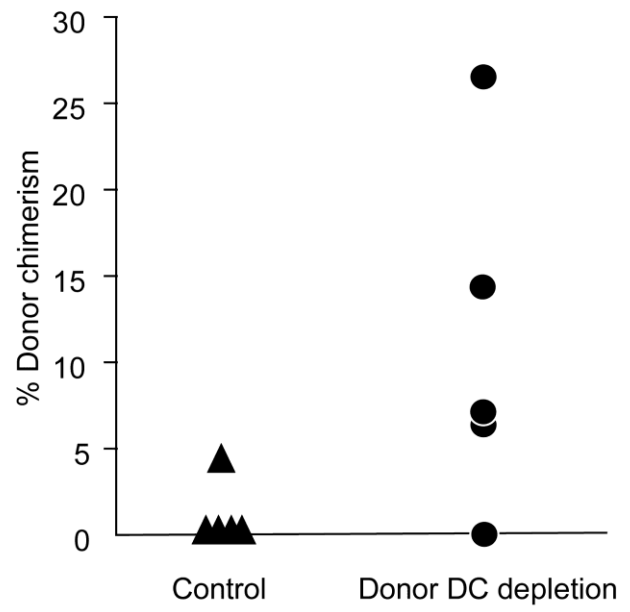


Figure 4

