High Doses of Purified Stem Cells Cause Early Hematopoietic Recovery in Syngeneic and Allogeneic Hosts

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Abstract

In humans, autologous transplants derived from bone marrow (BM) usually engraft more slowly than transplants derived from mobilized peripheral blood. Allogeneic BM transplants show a further delay in engraftment and have an apparent requirement for donor T cells to facilitate engraftment. In mice, Thy-1.1^{lo}Lin$^{-/lo}$Sca-1^{+} hematopoietic stem cells (HSCs) are the principal population in BM which is responsible for engraftment in syngeneic hosts at radioprotective doses, and higher doses of HSCs can radioprotect an allogeneic host in the absence of donor T cells. Using the mouse as a preclinical model, we wished to test to what extent engraftment kinetics was a function of HSC content, and whether at high doses of c-Kit^{+}Thy-1.1^{lo}Lin$^{-/lo}$Sca-1^{+} (KTLS) cells rapid allogeneic engraftment could also be achieved. Here we demonstrate that engraftment kinetics varied greatly over the range of KTLS doses tested (100–10,000 cells), with the most rapid engraftment being obtained with a dose of 5,000 or more syngeneic cells. Mobilized splenic KTLS cells and the rhodamine 123^{lo} subset of KTLS cells were also able to engraft rapidly. Higher doses of allogeneic cells were needed to produce equivalent engraftment kinetics. This suggests that in mice even fully allogeneic barriers can be traversed with high doses of HSCs, and that in humans it may be possible to obtain rapid engraftment in an allogeneic context with clinically achievable doses of purified HSCs. (*J. Clin. Invest.* 1998. 101:961–966.)
Key words: hematopoietic stem cell transplantation • hematopoiesis • mobilized peripheral blood

Introduction

The use of mobilized peripheral blood (MPB)[1] transplants in place of bone marrow (BM) has reduced the time required for engraftment (1). As a result, patients suffer fewer infections, require fewer platelet transfusions, and leave the hospital earlier (1). The reduction in engraftment time could be attributed to many factors, including mobilization of nonstem cell progenitors, or mobilization of increased numbers of hematopoietic stem cells (HSC), or both. Using a mouse model, we demonstrated previously that BM HSC, defined by the phenotype Thy-1.1^{lo}Lin$^{-/lo}$Sca-1^{+} (TLS) and c-Kit^{+}Thy-1.1^{lo}Lin$^{-/lo}$Sca-1^{+} (KTLS), were responsible for radioprotection and long-term multilineage reconstitution (2–4). KTLS BM cells include long-term multilineage reconstitution HSC, short-term HSC, multipotent progenitors, and some lymphoid progenitors (4). We previously transplanted 10^6 BM cells (which contain $\sim$ 500 TLS cells) in comparison with 500 purified TLS cells, and the recovery kinetics of white blood cells (WBC), platelets, and hematocrit were essentially indistinguishable (5). Here we transplanted higher doses of BM KTLS cells or mobilized KTLS cells; these high doses lead to dramatically reduced engraftment times. Even a primitive HSC subset was capable of rapid engraftment. These results contradict the notion that purified HSC are incapable of early engraftment, and provide a basis for understanding the efficacy of MPB transplants.

In humans, T cell–depleted BM transplants often fail to engraft across minor or major histocompatibility barriers. In the mouse, pure Thy-1.1^{lo}Lin$^{-/lo}$Sca-1^{+} allogeneic BM cells can engraft, but the protective dose for 95–100% of mice (PD95) was 10–50 times the usual dose for syngeneic transplants. To test whether high-dose allogeneic KTLS transplants could both engraft and contribute to early appearance of WBC and platelets, we transplanted 10^3–10^4 KTLS cells from C57Bl/Ka (H-2^b) into fully allogeneic BALB/c (H-2^d) hosts; at higher doses, there was rapid engraftment without indication of graft-versus-host disease. A comparison of the dose-dependent engraftment kinetics of syngeneic versus allogeneic KTLS cells revealed that a 10-fold barrier existed for radioprotection, and beyond that barrier the dose–response kinetics of engraftment for allogeneic HSC is less. These results provide preclinical evidence that purified HSC transplants can be used for syngeneic (autologous) and allogeneic transplants with rapid and sustained engraftment and without graft-versus-host disease.

Methods

Mouse strains and mobilization. The C57Bl/Ka-Thy-1.1 (H-2^b, Thy-1.1, Ly-5.2), C57Bl/Ka (H-2^b, Thy-1.2, Ly-5.2), and C57Bl/Ka-Thy-1.1 Ly-5.1 (H-2^b, Thy-1.1, Ly-5.1) were bred and maintained in the ani-

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1. *Abbreviations used in this paper:* BM, bone marrow; HSC, hematopoietic stem cell; KTLS, c-Kit^{+}Thy-1.1^{lo}Lin$^{-/lo}$Sca-1^{+}; MPB, mobilized peripheral blood; PB, peripheral blood; PD95, protective dose for 95–100% of mice; RBC, red blood cells; RH123, rhodamine 123; TLS, Thy-1.1^{lo}Lin$^{-/lo}$Sca-1^{+}; WBC, white blood cells.

mal care facility at SyStemix (Palo Alto, CA). BALB/c mice (H-2^d, Thy-1.2, Ly-5.2) were purchased from Simonsen (Gilroy, CA) and Taconic Farms (Germantown, NY). For mobilization treatment, mice were injected intraperitoneally with cyclophosphamide (200 mg/kg) (Cytoxan; Bristol Myers-Squibb, Princeton, NJ). The next day, the mice were anesthetized and implanted with osmotic minipumps (Alza Corp., Palo Alto, CA), for continuous infusion of G-CSF (190 μg/kg/d) (Filgrastim; Amgen, Thousand Oaks, CA) for 7 d.

Purification of c-Kit$^+$Thy-1^{lo}Lin$^{-/lo}$Sca-1$^+$ cells. KTLS cells were isolated from normal BM or spleens after mobilization treatment. The antibodies used to remove cells with lineage markers included: RA3-6B2 for the B lineage marker B220; RM.-5 (CD2), GK. 1.5 (CD4), 53-7.3 (CD5), and 53.6.72 (CD8) for T cell markers; RB6-8C5 (GR-1) and M1/70.15.11.5 (Mac-1) for myelomonocytic markers; and TER-119 for erythrocytes. Antibodies specific for the lineage markers were obtained from PharMingen (San Diego, CA) and were detected with phycoerythrin-conjugated polyclonal anti–rat antibody (Caltag, South San Francisco, CA). The cells were incubated with biotinylated Sca-1 mAb. Sca-1$^+$ cells were positively selected using the MACS® magnetic bead system (Miltenyi Biotec, Auburn, CA). The positively selected cells were further stained with fluorescein–conjugated-19XE5 (Thy-1.1), allophycocyanin–conjugated-2B8 (c-Kit), and steptavidin Texas red (BioMeda, Foster City, CA). The labeled cells were analyzed and sorted with a three laser FACS® (Becton Dickinson, San Jose, CA). After sorting, the purity of KTLS cells was reanalyzed by flow cytometry. In a set of experiments, the sorted KTLS cells were incubated with 0.2 μg/ml of rhodamine 123 (Rh123) dye (Molecular Probes, Eugene, OR) for 30 min at 37°C. The cells were washed and incubated at 37°C for 40 min to allow efflux of the Rh123 dye. KTLS cells were further separated into Rh123^{lo} and Rh123$^{mid\text{-}hi}$ subsets. About 15% of the KTLS cells were in the Rh123^{lo} gate. Since the frequency of KTLS cells in BM is ~ 0.04–0.09% in C57Bl-Thy-1.1 mice, Rh123^{lo} KTLS cells represent ~ 0.006–0.01% of BM cells.

Stem cell transplantation and PB cell analysis. Mice were lethally irradiated 1 d before transplantation. For congenic transplants, C57Bl/Ka mice were exposed to 1,100 rads in two split doses of 550 rads with a 3-h interval; for allogeneic transplants, BALB/c mice were exposed to 900 or 950 rads in two split doses with a 3-h interval. The next day, sorted KTLS or KTLS subsets were injected intravenously into the retroorbital plexus. PB from the transplanted mice was obtained (250 μl/mice) from the retroorbital sinus. 4–10 mice from each group per time point were analyzed and in most cases, mice were bled at only one time point. WBC, platelet, and hematocrits were counted with a CELL-DYN 3500 (Abbott Diagnostics, Mt. View, CA) calibrated for mouse blood samples. The statistical significance of the differences of hematological parameters between different doses of HSC transplanted was determined by the two-tailed *t* test using StatView (Abacus Concepts, Berkeley, CA).

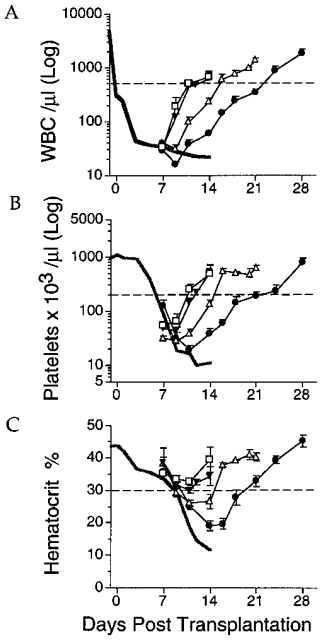

Figure 1. Hematopoietic recovery in lethally irradiated syngeneic mice transplanted with different cell doses of purified KTLS cells. Irradiated C57BL/Ka mice were injected with 100 (*closed circles*), 1,000 (*open triangles*), 5,000 (*closed triangles*), or 10,000 (*squares*) KTLS cells from normal BM. The purity of KTLS populations was 94–99% by reanalysis. The irradiation control mice were bled on days 0, 1, 3, 5, 7, 9, 11, 12, and 14 (*gray line*, n = 6–21 mice per point). WBC (*A*), platelet (*B*), and hematocrit (*C*) recovery kinetics are shown. The data represent the mean±SE obtained from 8 independent experiments with 4–10 mice/group in most cases. The dashed horizontal line represents recovery of blood levels to 500 WBC/μl (10% of preirradiation value), 200,000 platelets/μl (20% of preirradiation value), and 30% hematocrit (see Results). The statistical analyses were performed with data obtained from groups of mice that were injected with KTLS cells, as compared to controls that were not injected. WBC, no cells versus KTLS: 10,000 KTLS on day 9 ($P < 0.001$), 5,000 KTLS on day 9 ($P < 0.0001$), 1,000 KTLS on day 11 ($P < 0.0005$), and 100 KTLS on day 14 ($P < 0.0005$). Platelets, no cells versus KTLS: 10,000 KTLS on day 9 ($P < 0.0005$), 5,000 KTLS on day 9 ($P < 0.0005$), 1,000 KTLS on day 9 ($P < 0.01$), and 100 KTLS on day 14 ($P < 0.001$). Hematocrit, no cells versus KTLS: 10,000 KTLS on day 11 ($P < 0.0001$), 5,000 KTLS on day 11 ($P < 0.0001$), 1,000 KTLS on day 11 ($P < 0.0001$), and 100 KTLS on day 11 ($P < 0.001$). WBC, 1,000 vs. 5,000 KTLS on day 9 ($P < 0.001$), day 11 ($P < 0.0001$), and day 14 ($P < 0.005$). 100 vs. 1,000 KTLS on day 11 ($P < 0.05$), day 14 ($P < 0.0001$), day 16 ($P < 0.0005$), day 18 ($P < 0.001$), and day 21 ($P < 0.0001$). Platelets, 1,000 vs. 5,000 KTLS on day 11 ($P < 0.0001$) and day 14 ($P < 0.005$). 100 vs. 1,000 KTLS on day 11 ($P < 0.05$), day 14 ($P < 0.001$), day 16 ($P < 0.0001$), day 18 ($P < 0.005$), and day 21 ($P < 0.001$). Hematocrit, 1,000 vs. 5,000 KTLS on day 11 ($P < 0.005$) and 1,000 vs. 10,000 on day 14 ($P < 0.05$); 100 vs. 1,000 KTLS on day 14 ($P < 0.05$), day 16 ($P < 0.0005$), and day 18 ($P < 0.05$). There was no statistically significant difference in WBC, platelet, and hematopoietic recovery between 5,000 KTLS vs. 10,000 KTLS transplanted throughout this study ($P > 0.05$).

Results

High doses of BM stem cells lead to rapid engraftment in syngeneic (CD45 congenic) hosts. Graded doses (100–10,000) of purified C57Bl/Ka-Thy1.1 KTLS cells were injected into lethally irradiated C57Bl/Ka mice, and we monitored the number of days it took for the level of cells in the blood to reach 10% (500 WBC/μl of blood) and 20% (200,000 platelets/μl of blood) of preirradiation values for WBC and platelets. Fig. 1 *A* shows a dose-dependent kinetic recovery of WBC: the mice had reconstituted 500 WBC/μl on day 22 with 100 KTLS cells (the PD95); on day 15 with 1,000 KTLS cells; and on day 11 with both the 5,000 and 10,000 cell doses. BM transplants in humans usually achieve a WBC count of 500 cells/μl, from 14–28 d after reconstitution, while MPB transplants can reach this level 9–11 d after transplant (1, 6, 7). 5,000 mouse KTLS cells reconstituted 200,000 platelets/μl by days 11–12, while the PD95 (100 KTLS) reached these levels at 22–23 d after transplant (Fig. 1 *B*). In fact, as early as 9 d after transplant, mice transplanted with 5,000–10,000 KTLS cells displayed significantly higher levels of WBC and platelets compared to mice receiving 100 or 1,000 KTLS cells. As shown in Fig. 1 *C*, the same pattern is repeated for the hematocrit levels; at high KTLS doses the hematocrit never drops below 30%. Since the KTLS cells represent ~ 0.04–0.09% of the cells in BM of C57Bl-Thy-1.1 mice, 5,000 KTLS cells would be contained in 5×10^6–10^7 cells. Host mice receiving 10^7 BM cells displayed recoveries of 856±55 WBC/μl and 426,000±82,000 platelets/μl on day 11, both of which are higher than engraftment with 5,000 KTLS cells.

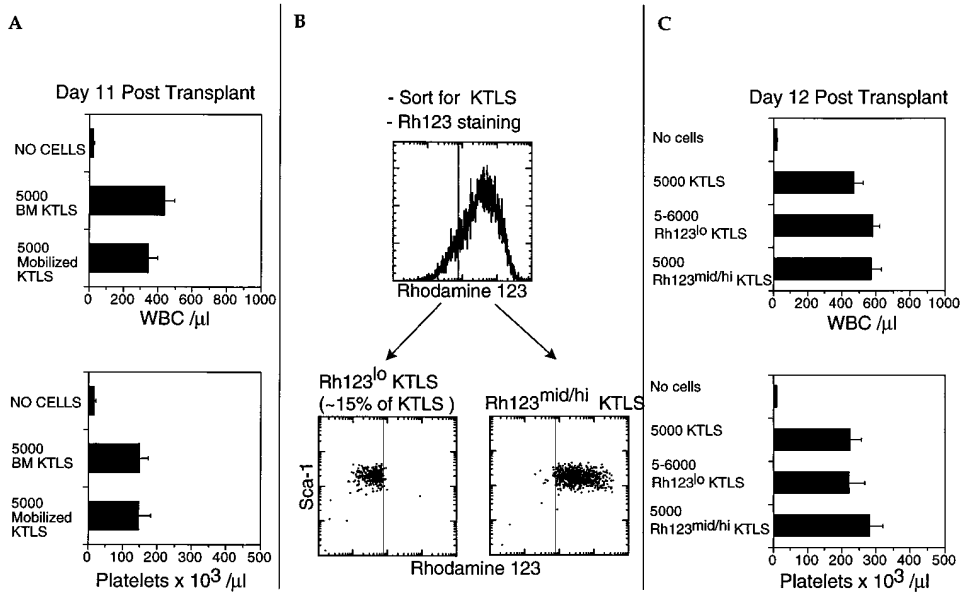

Figure 2. Transplantation of a high cell dose of mobilized KTLS and primitive Rh123^{lo} KTLS into lethally irradiated mice. Lethally irradiated mice were transplanted with either 5,000 normal BM KTLS or cyclophosphamide/G-CSF mobilized KTLS. Upon reanalysis, the purity of the KTLS population was 97% for BM and 95% for mobilized spleen. The data represent mean±SE of WBC, and platelet recovery on day 11 after transplant was shown (*A*). The difference between these two KTLS populations in reconstituting WBC and platelets is not significant ($P > 0.2$). The sorted KTLS cells from normal BM were further subdivided based on an Rh123 staining profile as shown in reanalysis (*B*). Lethally irradiated mice were transplanted with 5,000 KTLS, 5,000–6,000 Rh123^{lo} (96% pure on reanalysis), or 5,000 Rh123$^{mid\text{-}hi}$ KTLS cells (97% pure on reanalysis). WBC and platelet recoveries on day 12 after transplant are shown (*C*). Among the group receiving 5,000–6,000 Rh123^{lo} KTLS cells, 5,000 Rh123^{lo} KTLS cells gave rise to 523±95 WBC/μl and 196,000±40,000 platelets/μl ($n = 2$), while 6,000 Rh123^{lo} KTLS cells gave rise to 620±35 WBC/μl and 237,000±80,000 platelet/μl ($n = 4$). No statistically significant difference was seen in the recovery of WBC and platelets between 5,000 KTLS vs. 5,000–6,000 Rh123^{lo} KTLS, 5,000 KTLS vs. 5,000 Rh123$^{mid\text{-}hi}$ KTLS, and 5,000–6,000 Rh123^{lo} KTLS vs. 5,000 Rh123$^{mid\text{-}hi}$ KTLS cells ($P > 0.05$).

High doses of mobilized HSC also engraft rapidly. In mice, KTLS cells are mobilized to blood and spleen (8, 9). In Fig. 2 *A*, 5,000 mobilized spleen KTLS cells, as well as 5,000 BM KTLS cells contributed to WBC and platelet recovery by day 11. No statistically significant differences were observed in WBC, platelet, and hematocrit recovery between 5,000 KTLS cells, whether from normal BM or mobilized KTLS populations. Thus, mobilized HSC are approximately equivalent to BM HSC, and both are able to generate clinically relevant levels of blood elements by day 11.

Transplantation of high numbers of a primitive subset of HSC also leads to early engraftment. Rh123 is mainly excluded from a primitive subset of HSC (10), partly due to the action of the MDR-1 encoded P-glycoprotein, but also because this is a mitochondrial dye and Rh123^{lo} cells have fewer and/or less active mitochondria (11–16). It was of interest to test whether Rh123^{lo} KTLS cells could give rise to early engraftment, or whether engraftment would be delayed due to the need for these primitive HSC to mature into more developed progenitors. Therefore, 5,000 KTLS from BM were compared with either 5,000 Rh123$^{mid\text{-}hi}$ KTLS or 5,000–6,000 Rh123^{lo} KTLS subsets (Fig. 2 *B*). The results (Fig. 2 *C*) show that on day 12 after transplant the Rh123^{lo} subset performed as well as either the unfractionated or the Rh123$^{mid\text{-}hi}$ KTLS subset when WBC counts were analyzed (all > 400 WBC/μl). Similarly, the transplantation of 5,000–6,000 Rh123^{lo} KTLS cells reconstituted 220,000 platelets/μl on day 12, whereas the Rh123$^{mid\text{-}hi}$ subset and 5,000 unfractionated BM KTLS cells reconstituted 281,000 and 224,000 platelets/μl, respectively. Thus, high cell doses of the Rh123^{lo} HSC subset are equivalent to KTLS for early hematopoietic recovery. It is not yet known if rigorous selection of the most primitive LT-HSCs will show the same effect.

High doses of BM stem cells lead to rapid engraftment in allogeneic hosts. It was shown that 1,000–6,000 mouse TLS cells lacking detectable T cells can overcome barriers to transplantation of hematopoietic cells across several allogeneic strain combinations (17). In the following studies, purified C57Bl/Ka-Thy1.1 KTLS (H-2^{b}) cells were injected into lethally irradiated (900–950 rads) fully allogeneic BALB/c mice (H-2^{d}). This HSC-host strain combination requires stem cells to engraft across both minor and major histocompatiblilty barriers. As predicted, 100 allogeneic KTLS cells did not radioprotect 95% of the lethally irradiated BALB/c hosts, but a dose of 1,000 KTLS cells did provide radioprotection, as long as BALB/c mice were free from colonization with bacteria such as *Pasteurella pneumotropica* or β-strep group B, which can cause opportunistic infections after irradiation. When BALB/c mice were transplanted with 1,000 KTLS cells, donor-derived myeloid cells were detected in PB as early as 9 d after transplant, although the levels of WBC were still below 100 WBC/μl (Fig. 3 *A*). On day 14, virtually all myeloid cells were donor–derived (85±8% SE, $n = 4$). Donor-derived B cells, but not T cells, were detected as early as 17 d after transplant (Fig. 3 *B*). The appearance of donor-derived T cells was first detected at low levels 4 wk after transplant, and increased to normal levels thereafter. We tested long-term blood cell reconstitution in these animals. Long-term multilineage reconstitution is shown in Fig. 3 *D*; the majority of B, myeloid, and T cells were of donor origin 37 wk after transplant.

To test whether increasing the stem cell dose also resulted in a rapid engraftment of the allogeneic host, graded doses (1,000–10,000) of purified C57Bl/Ka-Thy1.1 KTLS cells were injected into lethally irradiated BALB/c mice, and the mice were monitored for the production of donor-derived cells. We

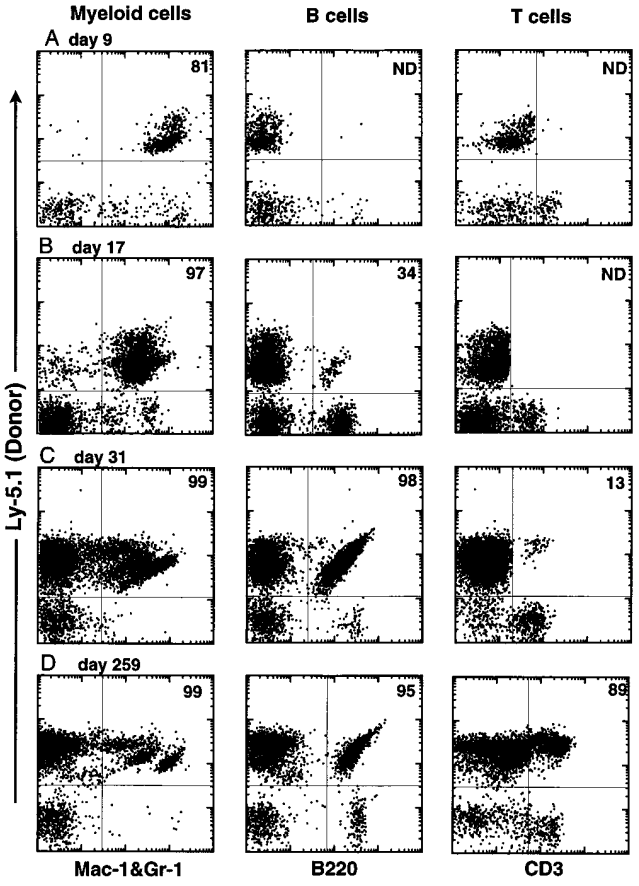

Figure 3. Hematolymphoid reconstitution of lethally irradiated allogeneic mice by transplanting purified 1,000 KTLS cells. BALB/c mice (H-2^d, Ly-5.2) were transplanted with 1,000 allogeneic KTLS cells (H-2^b, Ly-5.1). The PB cells from these mice were analyzed 9 d (*A*), 17 d (*B*), 31 d (*C*), and 259 d (*D*) after transplantation. Ly-5–marked (*Ly-5.1*) donor-derived myeloid (*Mac-1* and *Gr-1*), B (*B220*), and T (*CD3*) cells were determined by FACS® analysis. The percentage of Ly-5–marked donor cells in the Mac-1/Gr-1$^+$, B220$^+$, and CD3$^+$ cells originating from transplanted KTLS cells is indicated in each panel. On day 9, PB from two mice was combined for FACS® analysis, due to low WBC numbers.

found a similar pattern of dose-dependent kinetic recovery with both WBC and platelets. The level of WBC reached 10% of the preirradiation level (500 WBC/μl) more rapidly when the dose of KTLS cells was increased: on day 22 with the 1,000 cell dose; on day 16 with the 5,000 KTLS cell dose; and on day 11 with the 10,000 cell dose (Fig. 4 *A*). The highest cell dose, 10,000 KTLS cells, reconstituted 200,000 platelets/μl by 11–12 d after transplant, while mice which received the 5,000 and 1,000 KTLS cell dose reached these levels at 16 and 22 d, respectively (Fig. 4 *B*).

We compared the dose of stem cell that it takes to achieve delayed, intermediate, and rapid recovery of platelets and WBC for allogeneic and syngeneic transplants (Fig. 5). Delayed WBC and platelet recovery (i.e. > 21 d to reach 500 WBC/μl or 200,000 platelets/μl), occurred with 100 syngeneic and 1,000 allogeneic KTLS cells (Fig. 5, *A* and *E*). Intermediate WBC and platelet recovery (i.e., 14–21 d to reach 500 WBC/μl or 200,000 platelets/μl) occurred with 1,000 KTLS cells in syngeneic hosts, and 5,000 KTLS cells in allogeneic hosts (Fig. 5, *B* and *F*). Rapid WBC and platelet recovery in syngeneic mice could be achieved by 11 d with a dose of 0.5–1 × 10^4 KTLS cells, and by 11–12 d in allogeneic hosts with a dose of 10^4 KTLS cells (Fig. 5, *C*, *D*, *B*, and *H*); in fact, for WBC and platelets, both syngeneic and allogeneic hosts had similar recovery kinetics with a dose of 10^4 KTLS cells (Fig. 5, *D* and *H*). Fig. 6 summarizes the effect of KTLS cell dose in syngeneic versus allogeneic transplants, based on the number of days that are required to engraft 500 WBC/μl. Ten times more allogeneic cells were needed at radioprotective doses to produce equivalent engraftment kinetics, while only two times more cells were required in dose ranges giving the most rapid engraftment. Thus, not only can allogeneic barriers be overcome by increasing the HSC dose, but once the allogeneic barrier has been breached, the dose-dependent kinetics of recovery for allogeneic and syngeneic KTLS cells are similar (Fig. 5, *D* and *H*).

Discussion

These results show that in both the syngeneic and allogeneic setting, HSC can dramatically shorten engraftment times when

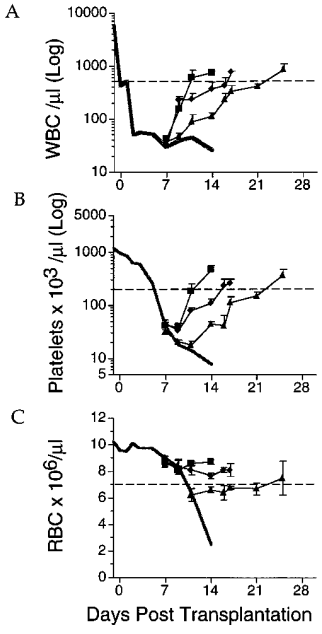

Figure 4. Hematopoietic recovery in lethally irradiated allogeneic mice transplanted with different cell doses of purified KTLS cells. Irradiated BALB/c mice were injected with 1,000 (*triangles*), 5,000 (*diamonds*), or 10,000 (*squares*) KTLS cells from normal BM. Irradiation control mice were bled on days 0, 1, 3, 5, 7, 9, 11, 12, and 14 (*gray line*, n = 3–9 per time point). WBC (*A*), platelet (*B*), and red blood cells (RBC) (*C*) recovery kinetics were shown. Hematocrit values were not available due to changes in instrumental set up. The data represent the mean±SE obtained from 10 independent experiments with 4–10 mice/group in most cases. The horizontal line indicates 500 WBC/μl (10% of preirradiation value) or 200,000 platelets/μl (20% of preirradiation value). The statistical analyses were performed with data obtained from groups of mice that were injected with KTLS cells, as compared to control mice that were not injected. WBC, no cells versus KTLS: 10,000 KTLS on day 9 ($P < 0.05$), 5,000 KTLS on day 9 ($P < 0.0001$), and 1,000 KTLS on day 14 ($P < 0.005$). Platelets, no cells versus KTLS: 10,000 KTLS on day 9 ($P < 0.005$), 5,000 KTLS on day 9 ($P < 0.05$), and 1,000 KTLS on day 14 ($P < 0.0005$). RBC, no cells versus KTLS: 10,000 KTLS on day 11 ($P < 0.05$), 5,000 KTLS on day 14 ($P < 0.0001$), and 1,000 KTLS on day 14 ($P < 0.0001$). WBC, 5,000 vs. 10,000 KTLS on day 14 ($P < 0.05$). 1,000 vs. 5,000 KTLS on day 9 ($P < 0.005$), day 14 ($P < 0.005$), and day 17 ($P < 0.05$). Platelets, 5,000 vs. 10,000 KTLS on day 14 ($P < 0.005$). 1,000 vs. 5,000 KTLS on day 11 ($P < 0.005$), and day 14 ($P < 0.001$). RBC, 5,000 vs. 10,000 KTLS on day 14 ($P < 0.01$). 1,000 vs. 5,000 on day 11 ($P < 0.05$), day 14 ($P < 0.005$), day 16 ($P < 0.05$), and day 17 ($P < 0.01$).

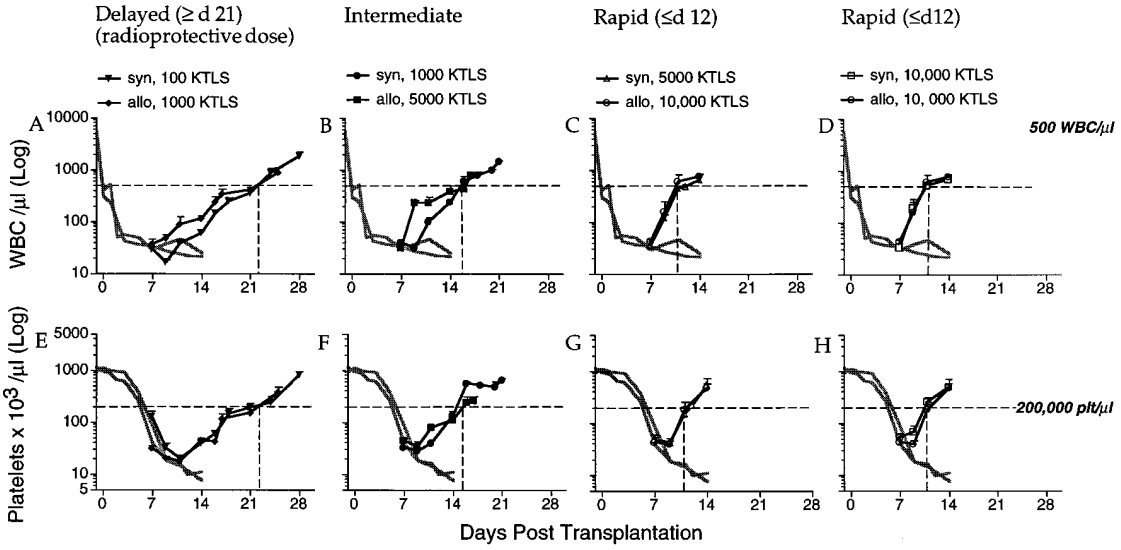

Figure 5. Comparison of stem cell dose required to achieve early hematopoietic recovery in syngeneic versus allogeneic hosts. Syngeneic or allogeneic mice were transplanted with purified KTLS cells as described in Figs. 1 and 4, respectively. The kinetics were categorized into delayed, intermediate, and rapid recovery for both WBC and platelets. The graphs show the hematopoietic recovery of syngeneic mice that were transplanted with 100 (*closed triangles*) (*A* and *E*), 1,000 (*closed circles*) (*B* and *F*), 5,000 (*open triangles*) (*C* and *G*), and 10,000 (*open squares*) (*D* and *H*) KTLS cells compared to recovery of allogeneic mice transplanted with 1,000 (*diamonds*) (*A* and *E*), 5,000 (*closed squares*) (*B* and *F*), and 10,000 (*open circles*) (*C*, *D*, *G*, and *H*) KTLS cells. The dashed horizontal line represents recovery of blood levels to 500 WBC/μl (10% of preirradiation value), and 200,000 platelets/μl (20% of preirradiation value).

given in high doses. Purified mouse BM HSC, mobilized KTLS HSC, and the Rh123^{lo} subset of HSC, all populations free of T cells, were able to achieve this rapid engraftment. There appears to be no significant difference in engraftment times when mobilized histocompatible KTLS HSC were compared with BM KTLS HSC or even the primitive Rh123^{lo} HSC subset. The level of possible contamination of these cells with other cells, for example, multilineage progenitors, was 1–6% at the 5,000 and 10,000 KTLS dose: these are clearly insufficient levels to account for early engraftment (3). KTLS and Rh123^{lo} cells are devoid of day-8 CFU-S, but are highly enriched for day-12 CFU-S, LTC-IC (CAFC), and up to 83% of these cells respond to a cocktail of cytokines by forming methylcellulose colonies (CFC); they do not respond to IL-3 alone, while most nonstem cell progenitors can (2, 4, 12, 18). Thus they are cells with a broad range of response potentials: they contain long-term HSC, short-term HSC, multipotent progenitors, a rare subset of CFC, and day-12 CFU-S. Although one can argue the definition of HSC, from a clinical point of view these rare KTLS cells are consistently able to generate a rapid and sustained engraftment. Nibley and Spangrude have independently characterized the engraftment ability of Thy-1.1^{lo} Lin^{-}Sca-1^{+}Rh123^{lo} and Thy-1.1^{lo}Lin^{neg}Sca-1^{+} Rh123^{hi} BM cells (19). In their study, 1,000 cells from both populations contributed equally to WBC recovery up to day 12. Mice transplanted with 1,000 Thy-1.1^{lo}Lin^{neg}Sca-1^{+}Rh123^{low} cells also provided sustained recovery of WBC levels, while transplants with 1,000 Thy-1.1^{lo}Lin^{neg}Sca-1^{+}Rh123^{high} cells only provided early, but not sustained WBC recovery (19), similar to the Mac-1^{lo} subsets of KTLS cells tested previously at the clonal level (4).

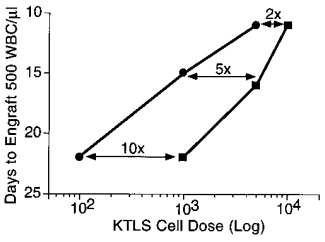

Figure 6. Effect of stem cell dose on syngeneic and allogeneic transplants. Syngeneic or allogeneic mice were transplanted with purified KTLS cells as described in Figs. 1 and 4, respectively. The dose of HSC and time that was required to achieve blood levels of 500 WBC/μl were compared in syngeneic (*circles*) and allogeneic (*squares*) hosts.

We propose that the principal advantage of MPB versus BM in autologous rapid engraftment is the higher HSC content of such transplants. While mobilization might also increase progenitors that are not fully multipotent, the major effect in congenic transplants can be achieved by increasing the KTLS dose by 50–100-fold over the PD95 (3). Dose–response studies of human purified HSC transplants will likely reveal the same sort of effect. Analysis of CD34^{+} cell numbers in transplants has shown that rapid engraftment is regularly achieved at $\geq 2 \times 10^6$ CD34^{+} cells/kg transplanted (6, 7, 20). Archimbaud et al., using MPB Thy-1^{+}CD34^{+}Lin^{-} HSC, reported rapid engraftment, i.e., ANC $>$ 500/μl on day 11 and platelet recovery on day 13 with 3×10^5–3×10^6 cells/kg (21).

These data confirm the importance of HSC content in transplantation into myeloablated hosts. In vitro expansion of hematopoietic cells from a defined number of input HSC can lead to a dramatic increase in myeloerythroid progenitors, but not yet to a net increase in HSC (22). When 1,000 normal BM TLS cells were cultured for 7 d in the presence of IL-3, IL-6, G-CSF, and SLF, there was a 2,062-fold expansion of total cell numbers and 83-fold expansion of CFCs. Nevertheless, CD45 congenic transplants of these expanded progenitors from 1,000

normal BM TLS HSC were no better at early or sustained reconstitution of lethally irradiated hosts than 1,000 TLS HSC from normal mice (22). In contrast, WBC and platelet recovery is delayed after transplanting Thy-1^{lo}Sca-1^{+}H2-K^{hi} HSC isolated from 5-FU–treated mice (5-FU HSC): the delay can be prevented by expanding 5-FU HSC ex vivo before transplantation (22). It is clear that improvements in expansion conditions will be required to increase their efficacy.

It has been reported that high doses of mouse TLS cells can overcome barriers to transplantation of hematopoietic cells across several allogeneic strain combinations (17, 23). In these cases, doses of HSC on the order of 3,000–6,000 HSC alone fully radioprotected and reconstituted lethally irradiated MHC-mismatched allogeneic hosts. (In the experiments reported here, a lower dose was required to radioprotect, presumably because KTLS fraction are more highly purified than the TLS fraction, and also because the hosts received 900–950 rads rather than the 800 rads in the previous experiments [17].) The doses of HSC that were required to radioprotect and fully reconstitute lethally irradiated allogeneic hosts (17) are comparable to the doses used in the Ly-5 congenic experiments reported here to accomplish rapid engraftment in congenic hosts. We tested the KTLS dose-dependent kinetics of early engraftment in allogeneic hosts. At the dose to achieve PD95 in these experiments (1,000 KTLS to allogeneic hosts; 100 to congenic hosts), the time to achieve 500 WBC/μl or 200,000 platelets/μl was $\sim$ 21 d. By increasing the number of KTLS cells per dose, the doses that it took to achieve equivalent engraftment kinetics in syngeneic/congenic versus allogeneic settings narrowed, up to a saturating dose of 10,000 KTLS cells wherein no differences in the kinetics to recovery of WBC or platelets could be detected. Thus, while a dose of 10–50 times the PD95 KTLS dose was required for rapid engraftment in the congenic model, rapid engraftment required at most a dose of 10 times the PD95 of KTLS in this fully allogeneic situation. These data indicate that the interaction between allogeneic KTLS (and their progeny) with host barrier functions (cells) are quantitative and finite, and that much is yet to be learned about the nature of the interacting donor and host cells in terms of cell phenotypes, cell functions, the time after KTLS cell infusion when interactions are initiated and completed, and the anatomical sites wherein such interactions occur. But the major lesson from this preclinical model in terms of allogeneic BMT is that T cell–free donor–stem cell grafts within a dose range that allows early autologous engraftment might also lead to rescue and reconstitution of allogeneic hosts.

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