

Mobilization of Peripheral Blood Progenitor Cells by Betafectin® PGG-Glucan Alone and in Combination with Granulocyte Colony-Stimulating Factor

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ABSTRACT

Betafectin® PGG-Glucan, a novel β -(1,6) branched β -(1,3) glucan purified from the cell walls of *Saccharomyces cerevisiae*, has been shown to synergize with myeloid growth factors in vitro and to enhance hematopoietic recovery in myelosuppressed mice and primates. Here we report that PGG-Glucan is also capable of mobilizing peripheral blood progenitor cells (PBPC). PGG-Glucan (0.5 mg/kg to 16 mg/kg) was administered intravenously to C3H/HeN male mice and blood collected at times ranging from 30 min to seven days after injection. Based on granulocyte-macrophage colony-forming cell (GM-CFC) levels, peak mobilization occurred 30 min after a 2 mg/kg PGG-Glucan dose. At this time GM-CFC numbers in PGG-Glucan-treated mice were approximately fourfold greater than in saline-treated control mice. A second, smaller wave of GM-CFC mobilization (approximately twofold increase) also occurred on days 4 and 5 after PGG-Glucan treatment. Mobilization was not associated with the induction of α -chemokines, which have recently been reported to induce rapid progenitor cell mobilization. Competitive repopulation experiments performed in

irradiated female C3H/HeN mice revealed that, at three months after transplantation, more male DNA was present in bone marrow, splenic, and thymic tissues from animals transplanted with cells obtained from mice 30 min after a 2 mg/kg PGG-Glucan dose than in tissues from animals transplanted with cells obtained from saline-treated mice. Additional experiments evaluated the mobilization effects of PGG-Glucan (2 mg/kg) administered to mice which had been pretreated for three consecutive days with G-CSF (125 μ g/kg/day). When blood was collected 30 min after PGG-Glucan treatment, the number of GM-CFC mobilized in combination-treated mice was additive between the number mobilized in mice treated with G-CSF alone and the number mobilized in mice treated with PGG-Glucan alone. These studies demonstrate that: A) PGG-Glucan can rapidly mobilize PBPC; B) the kinetic pattern of PGG-Glucan-induced mobilization is different from that of the CSFs; C) the reconstitutive potential of PGG-Glucan mobilized cells is greater than that of steady-state PBPC, and D) PGG-Glucan can enhance G-CSF-mediated PBPC mobilization. *Stem Cells* 1998;16:208-217

INTRODUCTION

The use of peripheral blood progenitor cell (PBPC) transplantation in the treatment of malignancies continues to rapidly expand. As support for dose-intensive chemotherapy, autologous PBPC transplantation now challenges or surpasses autologous bone marrow transplantation, and allogeneic PBPC transplantation appears promising [1-5]. With

the development of PBPC transplantation, collection of steady-state PBPC (requiring as many as 7 to 10 pheresis procedures) has been replaced with collection of PBPC mobilized by chemotherapy, growth factors, and chemotherapy plus growth factors [1, 2, 5, 6]. PBPC enrichment over steady-state levels has been reported to be approximately 30-fold following chemotherapy alone or growth factors

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alone, and approximately 200-fold following chemotherapy plus growth factors [6]. Concomitant with the ability to enrich PBPC levels has been the ability to collect sufficient cells to ensure durable engraftment with a reduced number of pheresis procedures. Although mobilization following chemotherapy plus growth factors can reduce PBPC collection to a single pheresis, in circumstances of allogeneic transplantation, the administration of chemotherapy to normal donors is not a feasible option. In these instances, attempts are being made to improve PBPC collection using intensive growth factor treatment. In spite of these improvements, pheresis-related catheter thrombosis and organ-specific complications related to the administration of mobilizing chemotherapy and/or growth factors are often still clinical concerns [6, 7]. In light of this, there exists a need to explore alternative methods for optimizing PBPC mobilization.

Yeast-derived β -glucans is a novel class of carbohydrate compounds which have been shown to exert potent hematopoietic and anti-infective activity in normal and myelosuppressed animals following single-dose administration [8-15]. In mice, a single administration of particulate β -glucan has also been demonstrated to mobilize granulocyte-macrophage colony-forming cells (GM-CFC) and spleen colony-forming units (CFU-S) [9, 10]. Although early β -glucan preparations were particulate in nature [8-11], derivatized (e.g., aminated, sulfated, phosphorylated) soluble β -glucans have subsequently been prepared, and some of these β -glucans shown to also possess potent hematopoietic and anti-infective activity [12-15]; the mobilizing capacity of soluble β -glucans, however, was not evaluated.

In part, development of derivatized soluble β -glucans has been dampened by the likelihood of undesirable clinical side effects related to their strong cytokine-inducing activities [12, 15-20]. Betafectin[®] PGG-Glucan is a novel, nonderivatized, β -(1,6) branched β -(1,3) glucan isolated from the cell walls of a proprietary strain of the yeast *Saccharomyces cerevisiae*. It is completely soluble in aqueous solutions within the physiologic pH range and has a defined size range of $170,000 \pm 20,000$ daltons [21, 22]. PGG-Glucan has been shown to possess broad anti-infective activities without the induction of proinflammatory cytokines [21, 23-25]. Currently, PGG-Glucan is being evaluated for the ability to reduce postsurgical infections in a phase III clinical trial.

PGG-Glucan has also been shown to possess hematopoietic activities. In vitro PGG-Glucan synergizes with myeloid growth factors to enhance hematopoietic cell proliferation, and in vivo it has been demonstrated to enhance hematopoietic recovery in myelosuppressed mice and primates [26-28]. The purpose of these studies was to evaluate the ability of PGG-Glucan to mobilize PBPC in normal mice. Both the effects of PGG-Glucan alone and the effects of PGG-Glucan

in combination with G-CSF were evaluated. The results indicate not only that a single administration of PGG-Glucan can rapidly mobilize PBPC capable of long-term hematopoietic reconstitution but also that PGG-Glucan can enhance the PBPC mobilization produced by G-CSF administration.

MATERIALS AND METHODS

Seven-week-old C3H/HeN female and male mice were purchased from Taconic Farms (Germantown, NY). Mice were barrier-reared and reported by the supplier to be free of adventitious viruses and pathogenic organisms. Animals were housed 10 per group in plastic Micro-Barrier cages (Allentown Caging Equipment; Allentown, NJ) on hardwood-chip contact bedding and were provided food and chlorinated water ad libitum. Mice were allowed to acclimate to the environment of the Alpha-Beta Technology animal facility for one week prior to being entered into experimental protocols that were approved by the Institutional Animal Care and Use Committee.

Male mice were treated with PGG-Glucan (Betafectin[®]; Alpha-Beta Technology, Inc.; Worcester, MA), recombinant human G-CSF (Amgen; Thousand Oaks, CA) or pyrogen-free saline (McGaw, Inc.; Irvine, CA). PGG-Glucan was administered i.v. at single doses ranging from 0.5 mg/kg to 16 mg/kg. G-CSF was administered s.c. on three consecutive days at a dose of 125 μ g/kg/d. Blood was collected by cardiac puncture from mice under isoflurane (AErrane[®]; Ohmeda PPD; Liberty Corner, NJ) anesthesia at times ranging from 30 min to seven days after treatment. Depending on the time point evaluated and the number of cells needed for specific studies, blood was collected from three to nine mice per treatment group and pooled for further processing. In some instances, femurs and spleens were also removed to prepare cell suspensions as previously described [12]. Peripheral white blood cell (WBC), red blood cell (RBC), and platelet (PLT) counts were determined using a Sero System 9010 Hematology Analyzer (Biochem Immunosystems; Allentown, PA) and light-density peripheral blood mononuclear cells (PBMC) were separated on LSM[®] (Organon Technika; Durham, NC) according to the manufacturer's instructions.

Bone marrow cells, spleen cells, and PBMC were cultured for GM-CFC in methylcellulose media (Stem Cell Technologies; Vancouver, Canada) in the presence of 0.1 ng/ml of recombinant murine GM-CSF (R&D Systems; Minneapolis, MN). Cultures were incubated at 37°C in 5% (v/v) CO₂ and colonies scored after seven days of culture. In some instances PBMC were also injected i.v. into irradiated female C3H/HeN mice to assay their short-term (two-week) and long-term (three-month) in vivo reconstitutive potential. Female recipient mice were exposed to 8.5 Gy of whole-body ¹³⁷Cs radiation administered at 0.9 Gy/min using a Gammacell-40 source (Atomic Energy of Canada; Ottawa, Canada).

Reconstititional analysis was performed as previously described [29]. Briefly, the femurs, spleen, and thymus were removed from individual mice and DNA-extracted to prepare Southern blots. Based on 260:280 ratios, equal amounts of DNA from each sample were loaded per gel and DNA analyzed for the presence of male Y-specific sequences using the pY2-cDNA probe (courtesy of *Dr. Ihor Lemischka*, Princeton University; Princeton, NJ). Blots were probed overnight at 65°C, and autoradiography was performed at -70°C for varying time intervals. Filters were then exposed to photostimulatable storage phosphorimaging plates (Molecular Dynamics; Sunnyvale, CA) and after scanning the plates with a 400A Phosphorimager (Molecular Dynamics), the percent of male and female DNA in each sample was quantified based on comparison to a male murine DNA standard. DNA calculations were normalized based on verification of the amount of DNA loaded for each sample. As previously described [29-30] this was done by reprobing membranes with a cDNA probe for interleukin 3 (IL-3; courtesy of *Dr. James Ihle*, DNAX; Palo Alto, CA) and also quantifying by phosphorimage analysis.

Serum samples for chemokine analysis were collected at 0.25, 0.5, 1, 2, and 3 h after i.v. administration of 2 mg/kg of PGG-Glucan or 500 µg/kg of *E. coli* 0127:B8 lipopolysaccharide (LPS; Calbiochem; La Jolla, CA). The α-chemokines macrophage inflammatory protein-2 (MIP-2) and KC were quantitated by enzyme-linked immunosorbent assay (ELISA) using commercial kits specific for the detection of these murine proteins (R&D Systems).

Unless stated otherwise results are presented as the mean ± standard error (SE) of data obtained in replicate experiments. Statistical analyses were performed by analysis of variance or two-tailed *t*-test using Instat® software (GraphPad; San Diego, CA).

RESULTS

Mobilization with PGG-Glucan Alone

The ability of a single 2-mg/kg dose of PGG-Glucan to mobilize GM-CFC into the peripheral circulation of mice is illustrated in Figure 1. Peak mobilization occurred within 30 min of PGG-Glucan administration, at which time approximately a fourfold increase in GM-CFC numbers was observed. Although by day 2, GM-CFC mobilization appeared to subside, a second, smaller wave of mobilization occurred on days 4-6. Both the early (Fig. 2A) and late (Fig. 2B) waves of GM-CFC mobilization were dose-dependent, with the 2 mg/kg PGG-Glucan dose inducing optimal effects. Throughout the time course studied, bone marrow GM-CFC numbers remained within control levels (Table 1). Consistent with a lack of hematopoietic effects in the bone marrow, peripheral WBC, RBC, and PLT numbers

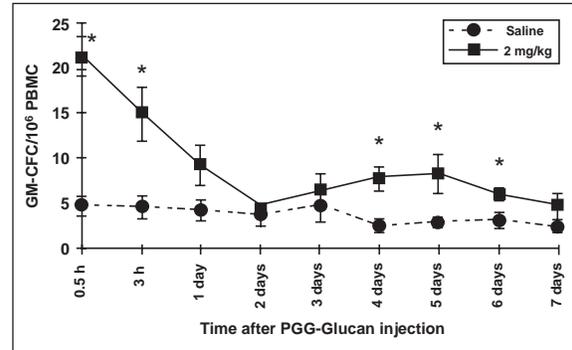


Figure 1. Time course of GM-CFC mobilization in C3H/HeN male mice injected i.v. with either saline or 2 mg/kg of PGG-Glucan. Mean ± SE of values obtained from at least three individual experiments at each time point. * *p* < 0.05, with respect to saline.

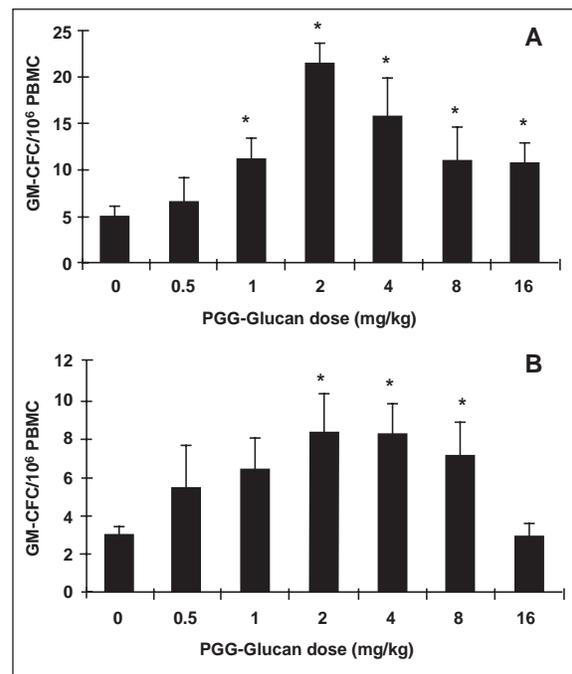


Figure 2. Dose response for PGG-Glucan-induced GM-CFC mobilization at the 30-min (A) and five-day (B) mobilization peaks. C3H/HeN male mice were injected i.v. with either saline or 0.5 mg/kg - 16 mg/kg of PGG-Glucan. Mean ± SE of values obtained from at least three individual experiments at each dose. * *p* < 0.05, with respect to saline.

in PGG-Glucan-treated mice fluctuated within control levels with the exception of a slight increase in RBC numbers on day 1 and a slight increase in RBC and PLT numbers on day 4 (Table 1). The short-term and long-term reconstititional potential of cells mobilized by PGG-Glucan is illustrated in Figure 3. In these studies lethally-irradiated female mice were transplanted with 3.4×10^6 PBMC isolated from normal

Table 1. PGG-Glucan treatment does not alter bone marrow GM-CFC proliferation or peripheral WBC, RBC, and PLT counts^a

	Time after Injection								
	0.5 h	3 h	1 day	2 days	3 days	4 days	5 days	6 days	7 days
GM-CFC per 10⁵									
Marrow cells									
Saline	54 ± 2	NA ^b	51 ± 3	45 ± 4	40 ± 3	28 ± 3	42 ± 4	41 ± 4	28 ± 2
PGG-Glucan	54 ± 3	NA ^b	66 ± 9	51 ± 3	39 ± 3	39 ± 7	43 ± 4	38 ± 7	35 ± 1
WBC/μl (× 10³)									
Saline	2.96 ± 0.16	2.64 ± 0.56	3.13 ± 0.42	2.98 ± 0.57	1.96 ± 0.19	2.66 ± 0.35	2.42 ± 0.33	2.55 ± 0.42	1.78 ± 0.23
PGG-Glucan	2.89 ± 0.17	2.52 ± 0.44	2.34 ± 0.24	2.78 ± 0.55	2.26 ± 0.15	2.41 ± 0.19	2.22 ± 0.37	2.01 ± 0.21	1.94 ± 0.17
RBC/μl (× 10⁶)									
Saline	7.25 ± 0.09	7.56 ± 0.21	7.40 ± 0.10	7.77 ± 0.22	7.73 ± 0.10	7.74 ± 0.06	7.78 ± 0.13	7.82 ± 0.12	7.93 ± 0.11
PGG-Glucan	7.29 ± 0.07	7.52 ± 0.09	7.87 ± 0.07 ^c	7.76 ± 0.24	7.65 ± 0.10	8.21 ± 0.12 ^c	7.88 ± 0.12	7.84 ± 0.14	8.06 ± 0.12
PLT/μl (× 10³)									
Saline	618 ± 13	574 ± 34	641 ± 25	673 ± 24	646 ± 13	665 ± 23	696 ± 20	684 ± 21	716 ± 24
PGG-Glucan	617 ± 13	650 ± 18	701 ± 13	661 ± 30	651 ± 22	758 ± 10 ^c	723 ± 23	735 ± 19	739 ± 22

^aEffects of saline or 2 mg/kg of PGG-Glucan (i.v.) on bone marrow GM-CFC proliferation and peripheral WBC, RBC and PLT counts in C3H/HeN male mice. Mean ± SE of values obtained from two to three GM-CFC experiments and 2-14 blood cell count experiments.

^bNot assayed.

^c $p < 0.05$, with respect to saline.

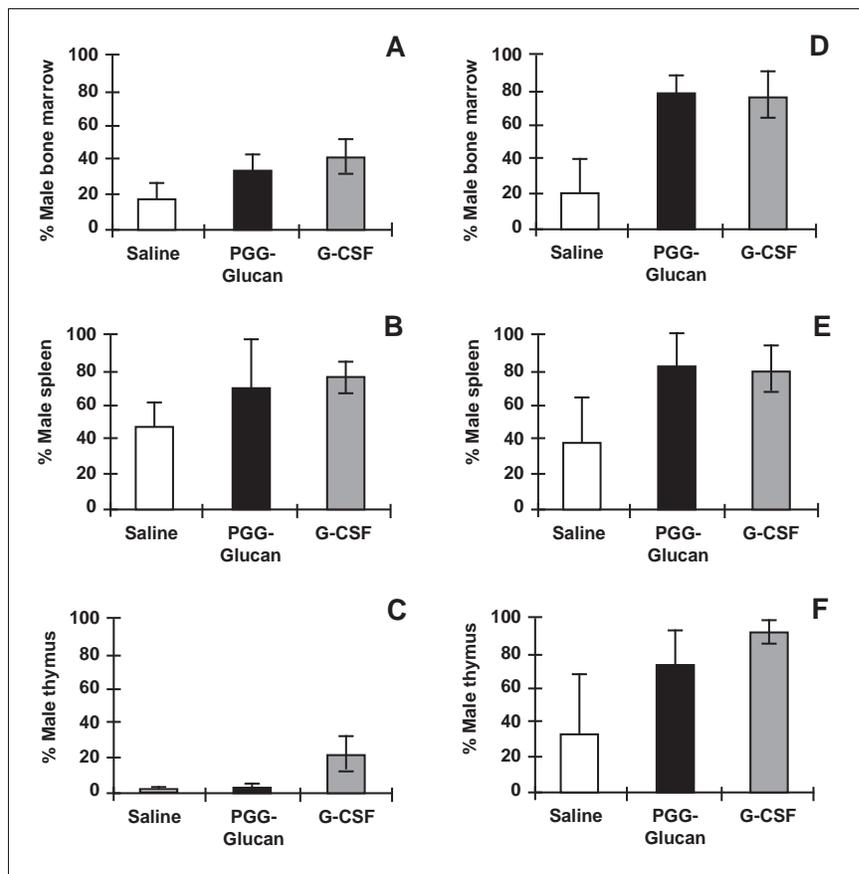


Figure 3. Short-term (two-week; A-C) and long-term (three-month; D-F) bone marrow, splenic and thymic competitive reconstitution produced by mobilized PBPC. Lethally irradiated female C3H/HeN recipient mice were transplanted with 3.4×10^6 normal C3H/HeN female PBMC along with 3.4×10^6 male C3H/HeN PBMC obtained from saline-treated mice, mice injected i.v. 30 min previously with 2 mg/kg of PGG-Glucan, or mice injected s.c. for three consecutive days with 125 μg/kg/d of G-CSF. The percent male-derived reconstitution was determined by Southern blot analysis with a Y-chromosome specific probe as described in **Materials and Methods**. Mean ± SE of values obtained from three individual mice per group.

female mice along with 3.4×10^6 PBMC isolated from male mice injected 30 min previously with either 2 mg/kg of PGG-Glucan or saline. Based on preliminary experiments, the

3.4×10^6 PBMC cell dose was chosen for these studies because it represented the average PBMC number in 1 ml of normal mouse blood and should insure the long-term survival

of all animals. All mice transplanted with cells at this dose survived until euthanized at two weeks or three months after transplantation to assay the reconstitutive potential of the male donor cells based on their ability to competitively repopulate the bone marrow, spleen, and thymus of the female recipients. Cells mobilized by PGG-Glucan tended to sustain both better short-term (Fig. 3A-3C) and long-term (Fig. 3D-3F) reconstitution than cells obtained from saline-treated mice. The enhanced reconstitution observed with PGG-Glucan-mobilized cells was more pronounced at three months than at two weeks. In the same experiment, the short-term and long-term competitive repopulation potential of 3.4×10^6 cells mobilized by three consecutive daily injections of G-CSF was evaluated. In all tissues examined, cells mobilized by G-CSF also produced better short-term and long-term reconstitution than cells obtained from saline-treated mice (Fig. 3). The two-week and three-month bone marrow and splenic reconstitution obtained with cells mobilized by PGG-Glucan was comparable to that obtained with cells mobilized by G-CSF. Early thymic reconstitution, however, was better with G-CSF-mobilized cells.

With respect to the competitive repopulation data presented in Figure 3, 50% male and 50% female repopulation would theoretically be expected in the organs of control mice transplanted with a 1:1 ratio of female:male cells. However, only 20% male (80% female) repopulation was observed in the bone marrow of control animals. In our studies, an 8.5-Gy radiation dose was used to prepare recipients for transplantation. This was the highest usable radiation dose based on preliminary studies revealing that higher doses induced death within four to six days due to gastrointestinal radiation injury. Although the 8.5-Gy radiation dose was severely myeloablative, it is likely that it was not entirely myeloablative. Thus, residual female host cells would increase the female:male cell ratio and skew towards female repopulation. Interestingly, however, control splenic reconstitution did approximate 50% male and 50% female. It has previously been demonstrated that, related to cell cycling differences, splenic GM-CFC are approximately twice as radiosensitive as bone marrow GM-CFC [30]. Based on this, the preparative radiation regimen would be expected to ablate a greater percentage of host splenic progenitors than host marrow progenitors. Such

a differential myeloablation prior to transplantation would result in a 1:1 female:male cell ratio in the spleen (yielding equal male and female reconstitution) but greater than a 1:1 female:male cell ratio in the marrow (favoring female reconstitution). In spite of these curiosities related to the preparative radiation treatment, they have no influence on the interpretation of our data since all animals were transplanted with exactly the same number of cells from saline-, PGG-Glucan- or G-CSF-treated mice, making relative comparisons of the reconstitutive potential of each of the donor populations still valid.

Because the α -chemokine interleukin 8 (IL-8) [31-33] has recently been shown to rapidly mobilize PBPC in a manner similar to that observed following PGG-Glucan administration, the possibility that mobilization in PGG-Glucan-treated mice was occurring secondary to α -chemokine release was investigated. Although we could not specifically evaluate induction of IL-8 because IL-8 per se has not been identified in mice, two α -chemokines considered to be murine functional homologs of IL-8, MIP-2 and KC, were evaluated. The data presented in Table 2 show that although MIP-2 and KC levels dramatically increase in response to the LPS used as a positive control in these studies, PGG-Glucan did not induce either of these chemokines.

Mobilization with PGG-Glucan in Combination with G-CSF

Because the kinetics of PGG-Glucan-induced mobilization appeared to differ from the mobilization kinetics of classical growth factors, PGG-Glucan and G-CSF combination studies were carried out to evaluate potential additive or synergistic mobilization effects. In these studies, mice were treated on three consecutive days with G-CSF and administered PGG-Glucan 24 h after the last G-CSF injection; PBPC mobilization

Table 2. PGG-Glucan treatment does not alter serum α -chemokine levels^a

	Time after Injection				
	15 min	30 min	1 h	2 h	3 h
MIP-2 (pg/ml)					
Saline	< 7.8 ^b	< 7.8 ^b	< 7.8 ^b	< 7.8 ^b	< 7.8 ^b
PGG-Glucan	< 7.8 ^b	< 7.8 ^b	< 7.8 ^b	< 7.8 ^b	< 7.8 ^b
LPS	40.8 ± 3.3	472.9 ± 23.9	> 8000 ^c	> 8000 ^c	5029.3 ± 32.0
KC (pg/ml)					
Saline	62.7 ± 2.5	61.1 ± 1.8	51.5 ± 0.9	36.8 ± 2.4	61.7 ± 1.5
PGG-Glucan	60.1 ± 2.0	40.5 ± 1.9	35.9 ± 1.8	45.2 ± 3.5	55.8 ± 1.7
LPS	406.8 ± 8.2	3863.6 ± 59.9	> 8000 ^c	> 8000 ^c	> 8000 ^c

^aEffects of i.v. administered saline, PGG-Glucan (2 mg/kg), or LPS (500 μ g/kg) on serum MIP-2 and KC levels in C3H/HeN male mice as determined by ELISA analysis. Sera were tested in duplicate or triplicate wells at 0, 1:2, 1:4, 1:8 and 1:16 dilutions. Data are expressed as the mean \pm SE of values from all dilutions yielding readings on standard curves generated with recombinant murine MIP-2 or KC.

^bLower limit of detection was 7.8 pg/ml for MIP-2 and 15.6 pg/ml for KC.

^cMaximum limit of detection based on the dilution scheme used.

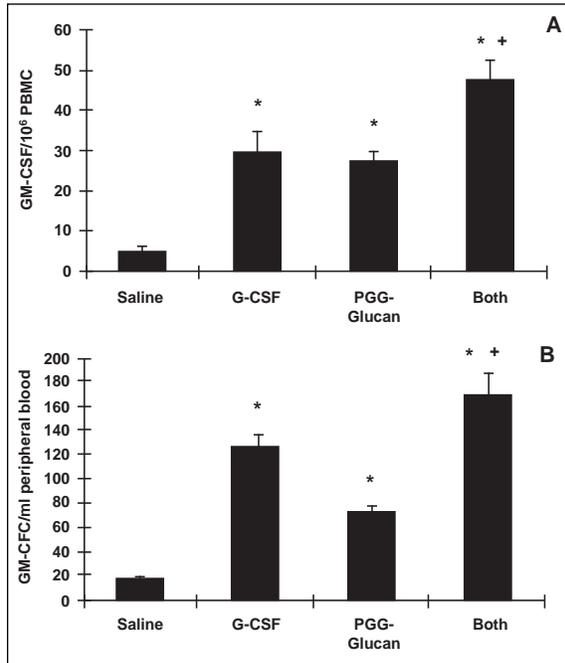


Figure 4. Effect of PGG-Glucan on mobilization of GM-CFC in mice pretreated with G-CSF. Blood was collected from C3H/HeN male mice 24 h after the third of three daily s.c. injections of G-CSF (125 μ g/kg/d), 30 min after a single i.v. injection of PGG-Glucan (2 mg/kg), or 30 min after a single injection of PGG-Glucan administered 24 h after the third of three daily G-CSF injections. Mean \pm SE of values obtained from two individual experiments. * $p < 0.05$, with respect to saline; + $p < 0.05$, with respect to G-CSF.

was determined 30 min following the PGG-Glucan treatment. Controls consisted of mice receiving G-CSF for three consecutive days followed by saline treatment 24 h later, mice receiving saline for three consecutive days followed by PGG-Glucan treatment 24 h later, and mice receiving saline at all treatment times. Alone, PGG-Glucan and G-CSF increased the concentration of GM-CFC per 10^6 PBMC to approximately equal levels (Fig. 4A); however, because WBC counts were higher in mice treated with G-CSF than in mice treated with PGG-Glucan, GM-CFC numbers per ml of blood were greater in G-CSF-treated mice than in PGG-Glucan-treated mice (Fig. 4B). GM-CFC mobilization in the combination-treated mice was approximately additive between that observed in mice treated with G-CSF alone and that observed in mice treated with PGG-Glucan alone whether evaluated as GM-CFC concentration per 10^6 PBMC or as GM-CFC numbers per ml of blood (Fig. 4). Growth-factor-induced PBPC mobilization has previously been shown to occur concomitantly with active progenitor cell proliferation. To evaluate mobilization mechanisms in the combination-treated mice, bone marrow, and splenic GM-CFC progenitor cell, activity was also evaluated in

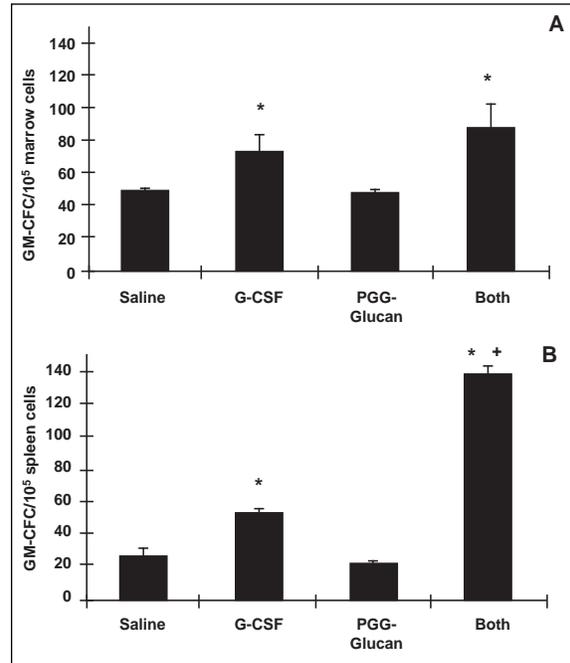


Figure 5. Effect of PGG-Glucan on bone marrow (A) and splenic (B) GM-CFC proliferation in mice pretreated with G-CSF. Bone marrow and spleen cells were prepared from C3H/HeN male mice 24 h after the third of three daily injections of G-CSF (125 μ g/kg/d), 30 min after a single injection of PGG-Glucan (2 mg/kg) or 30 minutes after a single injection of PGG-Glucan administered 24 h after the third of three daily G-CSF injections. Mean \pm SE of values obtained from two individual experiments. * $p < 0.05$, with respect to saline. + $p < 0.05$, with respect to G-CSF.

these animals. As expected, mobilization in G-CSF-treated mice was accompanied by an increase in bone marrow GM-CFC numbers; however, no further GM-CFC increase was observed in the bone marrow of combination-treated mice (Fig. 5A). Mobilization in G-CSF-treated mice was also accompanied by an increase in splenic GM-CFC numbers (Fig. 5B). Interestingly, combination-treated mice did exhibit greater splenic GM-CFC numbers than mice treated with G-CSF alone (Fig. 5B).

DISCUSSION

Laboratory and clinical investigations over the past several decades have clearly established that PBPC transplantation can bring about stable, complete, and long-lasting engraftment. PBPC are less painful to collect than bone marrow, appear to engraft more rapidly than bone marrow stem cells, and may be less contaminated with tumor cells. Although during steady-state hematopoiesis only small numbers of PBPC circulate in the blood, the advantages of PBPC transplantation have spurred interest in optimizing modalities that can safely and predictably increase PBPC numbers. Improved technologies

have rapidly developed to mobilize, collect, and cryopreserve PBPC for use in outpatient settings.

Although many growth factors and cytokines have been evaluated for PBPC mobilization potential, currently only GM-CSF and G-CSF have been approved as mobilizing agents by the Food and Drug Administration. The typical clinical G-CSF mobilization regimen consists of a five- to seven-day course of G-CSF (5 $\mu\text{g}/\text{kg}/\text{d}$ to 16 $\mu\text{g}/\text{kg}/\text{d}$) with PBPC collection pheresis being performed on days 5, 6, and 7. However, even in donors who are presumed to have normal bone marrow function, G-CSF administration elicits wide intersubject PBPC yields and poor yields in 5% to 15% of donors [34]. Similar effects have been observed with GM-CSF mobilization regimens. In light of such responses, efforts to develop better mobilizing regimens continue. Currently, the mobilizing potential of growth factor combinations as well as alternative cytokine regimens are under preclinical and clinical investigation. In this paper, we demonstrate the mobilizing potential of PGG-Glucan, a novel carbohydrate currently in clinical development as an anti-infective immunomodulatory agent.

As opposed to growth factor-induced PBPC mobilization which requires multiple daily growth factor treatments, peak PBPC mobilization occurred within 30 min following a single dose of PGG-Glucan. The PBPC mobilized by PGG-Glucan appeared to be qualitatively comparable to those mobilized by a three-day G-CSF regimen based on GM-CFC growth and long-term reconstitution potential (Figs. 3 and 4). Furthermore, presumably because of different mechanisms of action, administration of PGG-Glucan to mice pretreated with G-CSF augmented G-CSF-induced PBPC mobilization in an additive manner (Fig. 4).

Recently, a single-center, randomized, double-blind phase I PGG-Glucan mobilization study has also been performed. Nine normal, healthy, male volunteers were enrolled, with three each receiving PGG-Glucan at either 0.75 mg/kg, 1.50 mg/kg or 2.25 mg/kg administered as a single one-hour i.v. infusion. At times ranging from 2 h to 14 days after PGG-Glucan administration, blood samples were collected for cell counts and CD34⁺ analysis. This study showed a similar early increase (approximately threefold) in peripheral blood CD34⁺ cells within seven h of PGG-Glucan infusion followed by a second wave of mobilization on day 11 (Bleicher, P., Alpha-Beta Technology Inc., personal communication). The greatest response was observed with the 0.75-mg/kg PGG-Glucan dose. These data support the preclinical observations regarding the early mobilizing potential of PGG-Glucan reported in this paper.

PBPC mobilization following administration of particulate β -glucan has been previously reported [9-10]. The hematopoietic activities of particulate β -glucan, however, significantly differ from those of PGG-Glucan. In particular, administration of a single dose of particulate β -glucan results

in a dramatic stimulation of hematopoietic proliferation and amplification of bone marrow and splenic progenitor cell pools [9, 10] which does not occur following administration of PGG-Glucan. Furthermore, the peak PBPC mobilization observed following administration of particulate β -glucan has been reported to occur approximately five days after administration rather than immediately as seen following PGG-Glucan administration. Thus, the PBPC mobilization induced by PGG-Glucan administration is different from that induced by particulate β -glucan. With respect to soluble carbohydrates, previous studies have reported PBPC mobilization following administration of such agents [35-37]. In particular PBPC mobilization has been shown to occur within one to three h after i.v. administration of dextran sulfate (DS). Serious anaphylactic shock, which often occurred following systemic DS administration, however, limited its clinical development. Although the molecular weight of DS can affect its mobilization capacity, the mobilizing potential of DS has primarily been thought to be related to its polyanionic characteristic [36-37]. Because PGG-Glucan is essentially a neutral carbohydrate [21, 38-39], it can be inferred that PGG-Glucan induces mobilization via mechanisms different than those reported for DS.

Mobilization by PGG-Glucan also appears to occur via mechanisms different from growth factor-induced mobilization. Mobilization induced by growth factors has partially been attributed to an overall increase in the size of the progenitor cell pool subsequent to proliferative effects occurring after multiple doses of growth factor treatment [40-42]. This mechanism, however, cannot explain the mobilization observed following PGG-Glucan treatment since no increase in bone marrow or spleen progenitor cell numbers was observed within the short 30-min period between PGG-Glucan administration and blood collection (Table 1). The mere fact that PGG-Glucan induces a rapid PBPC mobilization suggests a redistribution of progenitors from the marrow to the peripheral blood rather than an amplification of progenitor cells. In the G-CSF and PGG-Glucan combination studies (Fig. 5), the increase in splenic GM-CFC numbers observed in combination-treated mice could also be explained by splenic trapping of mobilized marrow progenitors, i.e., a redistribution from the marrow to the spleen via the peripheral circulation.

It can be hypothesized that alterations in the progenitor/stroma environment must be occurring to facilitate the rapid PBPC redistribution observed following PGG-Glucan treatment. It is well known that carbohydrate-rich extracellular matrix molecules including collagens, glycoproteins, and glycosaminoglycans play a critical role in anchoring hematopoietic cells as well as providing local niches influencing their development [43]. Unlike other growth factor and cytokine-mobilizing agents, PGG-Glucan is a carbohydrate. If PGG-Glucan could in some manner competitively disrupt

carbohydrate-mediated anchoring of progenitor cells, it could be envisioned that progenitors would rapidly be peripheralized. The impact of adhesion molecules on mobilizing stem cells has recently been demonstrated in studies evaluating the mobilizing effects of anti-very-late-acting antigen-4 (VLA₄) treatment [44, 45]. In these studies, disruption of cytoadhesion by anti-VLA₄ administration resulted in rapid PBPC mobilization. Furthermore, some mobilizing cytokines such as *c-kit* ligand have been shown to alter adhesion molecule avidity [46]. Thus, it may also be possible that PGG-Glucan is capable of altering adhesion molecules such as VLA₄.

An additional mechanism through which PGG-Glucan may mediate PBPC mobilization could be through the induction of other factors known to produce rapid PBPC mobilization. Recently, the α -chemokine IL-8 [31-33] has been reported to mobilize PBPC within minutes of administration. Because of this, we evaluated whether PGG-Glucan was inducing α -chemokines. Since murine IL-8 per se has not been identified, we could not specifically evaluate induction of IL-8. However, we did evaluate effects on MIP-2 and KC which are α -chemokines known to bind to the murine IL-8 receptor and are considered to be murine functional homologs of IL-8 [47]. These studies revealed that serum MIP-2 and KC levels were not elevated following PGG-Glucan administration, suggesting that PGG-Glucan mobilizes PBPC in a manner independent of α -chemokine induction. The fact that PGG-Glucan mobilizes PBPC in a manner independent of α -chemokine induction is also suggested from the results of our PGG-Glucan and G-CSF combination studies (Fig. 4). In these studies, we observed enhanced PBPC mobilization in mice pretreated with G-CSF and subsequently administered PGG-Glucan, while *Laterveer et al.* reported decreased PBPC mobilization in mice pretreated with G-CSF and subsequently administered IL-8 [33]. Interestingly, BB-10010, a synthetic analog of the β -chemokine macrophage inflammatory protein 1- α (MIP-1 α), has also recently been reported to induce rapid PBPC mobilization [48]. Whether PGG-Glucan alters MIP-1 α production is not known at this time.

In analyzing the mobilizing potential of PGG-Glucan, we have attempted to quantitatively compare the mobilizing effects observed with PGG-Glucan with those induced by other cytokines and cytokine combinations. The PBPC-mobilizing abilities of numerous cytokines and cytokine combinations have been investigated in mice. However, these studies have utilized not only a variety of mouse strains and mouse models (e.g., normal mice, splenectomized mice, chemotherapy-treated mice), but also a variety of cytokine doses, administration routes, treatment durations, endpoint measurements (e.g., blood GM-CFC, CFU-S₈, granulocyte, erythroid, macrophage, megakaryocyte-CFC, CFU-S₁₁, long-term culture-initiating cells, radioprotection capability, reconstitution capability, etc.)

and data presentation formats (e.g., concentration per 10⁵ light-density blood cells, concentration per 10⁵ nucleated blood cells, concentration per ml of blood), making direct quantitative comparisons of the mobilizing efficacy of various agents somewhat complicated. In spite of these variables, we have attempted quantitative comparisons. Since our studies were performed in normal mice and used blood GM-CFC levels as a surrogate marker of PBPC mobilization, we have focused our comparisons on studies performed in normal mice in which effects on blood GM-CFC or/and CFU-S₈ levels have been reported. It should be noted that in making our comparisons, values for "fold-increases" in many instances have been estimated from graphically published results.

The most logical cytokines with which to compare the mobilizing potential of PGG-Glucan are MIP-1 α and IL-8 which, like PGG-Glucan, rapidly mobilize PBPC within 15-30 min after administration. The PBPC mobilization we observed after PGG-Glucan treatment was similar to *Lord's* reported threefold PBPC increase induced by the MIP-1 α analog BB-10010 (30 min after 2.5 μ g/mouse) [48] as well as to *Lui's* recently reported fourfold PBPC increase induced by IL-8 (15 min after 30 μ g/mouse) [49]. Interestingly, however, *Laterveer et al.* [31, 33] have reported 12- to 17-fold increases in blood GM-CFC levels 15 min after an identical 30 μ g/mouse IL-8 administration. The fact that these IL-8 studies were performed with different mouse strains may explain these response differences.

It is well known that high G-CSF doses administered for extended periods of time can significantly increase PBPC mobilization [41, 42, 50-53]. For our G-CSF/PGG-Glucan combination studies, however, we felt that an abbreviated G-CSF mobilization regimen would be sufficient for observing additive or synergistic mobilization effects. The three-day G-CSF administration protocol used in our combination studies was similar to the short, two-day courses of G-CSF administered in *Lord's* G-CSF/MIP-1 α combination studies [48] as well as *Laterveer's* G-CSF/IL-8 combination studies [33]. Alone, the G-CSF protocol used in our studies induced approximately a sixfold increase in peripheralized GM-CFC. In combination with PGG-Glucan, an approximate 10-fold increase was observed; this represented a 1.6-fold increase over the PBPC mobilization induced by G-CSF alone. This enhanced mobilization was comparable to the 1.5-fold increase over the PBPC mobilization induced by G-CSF alone in *Lord's* G-CSF/MIP-1 α combination studies [48]. Interestingly, our results contrasted those of *Laterveer et al.* in which they observed decreased PBPC mobilization in mice receiving combination G-CSF/IL-8 treatment compared with mice treated with G-CSF alone [33].

Other cytokines, when administered for extended periods of time, have also been demonstrated to result in significant

PBPC mobilization as well as to synergize with G-CSF in peripheralizing progenitors. The most notable cytokine combinations are stem cell factor (SCF) plus G-CSF [51, 52] and Flt3 ligand plus G-CSF [53]. For example, following a 10-day course of SCF (50 $\mu\text{g}/\text{kg}/\text{d}$) plus G-CSF (250 $\mu\text{g}/\text{kg}/\text{d}$), peripheral CFU-S₈ levels have been reported to be increased approximately fivefold over those observed with G-CSF treatment alone [52]. More recently, an approximate 35-fold increase over mobilization levels observed with G-CSF alone has been reported following a six-day course of Flt3 ligand (10 $\mu\text{g}/\text{mouse}/\text{d}$) plus G-CSF (10 $\mu\text{g}/\text{mouse}/\text{d}$) [53]. Compared with these extended cytokine combinations, PGG-Glucan appears to only modestly enhance the mobilizing effects of G-CSF. However, whether the administration of PGG-Glucan to mice treated with such cytokine combinations

would further increase mobilization via its distinct mobilizing mechanisms remains to be determined.

CONCLUSION

In conclusion, these studies have demonstrated the PBPC-mobilizing potential of a novel carbohydrate, PGG-Glucan, which appears to mobilize PBPC in a manner different than reported for other mobilizing agents. These studies have also demonstrated proof-of-principle that PBPC mobilization may be enhanced through the combined use of agents acting through different mobilizing mechanisms.

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