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# Comparison of the Effect of Orally Administered Soluble β-(1-3),(1-6)-D-Glucan and of G-CSF on the Recovery of Murine Hematopoiesis

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**Abstract.** *β-Glucans are branched fungal polysaccharide compounds with pleiotropic activating effects on cells of the immune and the hematopoietic system. In this study, the hematopoiesis-promoting effect of an orally administered soluble β-(1-3),(1-6)-D-glucan and of intravenously (i.v.) injected recombinant human granulocyte colony-stimulating factor (G-CSF/filgrastim) was tested in cyclophosphamide (CY)-conditioned mice. Both agents were administered for 5 consecutive days following treatment with CY. When G-CSF and the carbohydrate compound were co-administered, a small but non-significant increase of granulopoiesis compared to G-CSF alone was detected. β-Glucan alone failed to augment granulopoiesis in the peripheral blood of CY-treated mice. However, both G-CSF and β-glucan significantly enhanced the recovery of monocytes in the peripheral blood of leukopenic mice when orally administered as single agents. In conclusion, the present study provides further evidence of a stimulatory function of orally administered β-glucans on monocyte production and shows a weak additive effect on granulopoiesis when co-administered with G-CSF into leukopenic mice.*

Myeloid growth factors such as granulocyte colony-stimulating factor (G-CSF) and granulocyte macrophage CSF (GM-CSF) regulate the proliferation, differentiation and maturation of murine and human hematopoietic progenitor cells (HPC) into granulocytes or macrophages (1-3). G-CSF strongly enhances cell division of granulocyte precursor cells during maturation and accelerates the release of mature functional neutrophils from the bone marrow into the circulation. Furthermore, the production of monocytes is markedly increased by G-CSF (3). Almost 20 years ago, recombinant human methionylated G-CSF (r-met-hu G-CSF/filgastrim) was introduced into clinical practice to accelerate the recovery of hematopoietic activity of neutropenic cancer patients after chemotherapy, radiotherapy or transplantation with autologous or allogeneic peripheral blood progenitor cells (PBPCs) (4, 5).

In several studies, soluble and particulate β-glucans, branched polysaccharide compounds derived from yeast or fungal cell wall, were also shown to enhance hematopoiesis when delivered intraperitoneally (*i.p.*) or orally into healthy and myelosuppressed mice (6-12). Moreover, intravenously (*i.v.*) injected β-glucans synergized with G-CSF to mobilize PBPCs and to accelerate hematopoietic regeneration following radiation-induced myelosuppression (12, 13). β-Glucans stimulate hematopoiesis by binding to hematopoietic progenitor cells (HPCs) *via* the C-type lectin receptor dectin-1 or complement receptor (CR) 3 (CD11b/CD18, Mac-1) (14-16). Activation of HPCs and leukocytes from cord blood or the spleen by polymeric carbohydrate compounds subsequently leads to enhanced production and secretion of CSFs (6, 8, 17-19). Recent studies revealed that orally administered β-glucans are well tolerated by cancer patients and might also exert a beneficial effect on the patients' hematopoiesis during chemotherapy (20, 21). In the present study, we compared the hematopoietic growth-promoting effect of a yeast-derived β-(1-3),(1-6)-D-glucan and G-CSF in cyclophosphamide (CY) myelosuppressed mice.

## Materials and Methods

*Reagents.* CY was purchased from Baxter Oncology GmbH (Halle, Germany), lipopolysaccharide (LPS) was delivered by Difco Laboratories (Detroit, MI, USA) and recombinant human (rh)G-CSF (Lenograstim) was purchased from Aventis (France). Aqueous preparation of β-(1-3), (1-6)-D-glucan (20 mg/ml), derived from the inner cell wall of *Saccharomyces cerevisiae*, was obtained from Biotec Pharmacon (Tromsø, Norway). The endotoxin level was <0.05 EU/ml.

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*Animals*. Female BDF-1 mice (C57BL/6 × DBA/2) were purchased from Charles River (Sulzfeld, Germany), and were used between 7 and 9 weeks of age. The mice were maintained in the pathogen-free animal facility of epo GmbH (Berlin-Buch, Berlin) following institutional guidelines and with approval from the appropriate authorities.

*Myelosuppressive treatment and administration of β-glucan and G-CSF*. To induce myelosuppression, BDF1-mice received *i.p.* CY (200 mg/kg) on day zero. Subsequently, the mice (8 per group) received β-glucan daily (400 μg/mouse in 0.1 ml phosphate-buffered saline (PBS)) intragastrically, or subcutaneously (*s.c.*) G-CSF (125 μg/kg in 0.1 ml PBS), or both. A control group received 0.1 ml PBS intragastri-cally. All treatments started at day 0 and were maintained until day 5.

*Counting of white blood cells (WBCs)*. The total number of WBCs was determined from all mice at intervals using a semi-automated Coulter counter (Beckmann, Krefeld, Germany). In brief, 20 μl peripheral blood were taken from the retro-orbital area using glass capillaries in EDTA tubes (BD Biosciences, Heidelberg, Germany). A total of 12 μl EDTA blood was assayed using the Coulter counter and the following parameters were recorded: WBCs $\times 10^6$/ml; red blood cells (RBCs) $\times 10^9$/ml and platelets $\times 10^9$/ml.

*Differential blood count*. Quantification of leukocyte populations comprising neutrophil, basophil and eosinophil granulocytes, metamyelocytes, lymphocytes and monocytes was performed by their characteristic morphology using light microscopy. In brief, blood smears on slides were taken and stained with Giemsa according to standard procedures. Manual counting of a representative field containing 100 cells was performed by two investigators blinded to the treatment groups. The absolute numbers of the different leukocyte populations within the blood was calculated by multiplication of their respective cell numbers with the WBC count.

*Statistical analysis*. Data are presented as mean±standard deviation (SD). Student's *t*-test served for statistical analysis of the data. A *p*-value less than 0.05 was considered to indicate a statistically significant difference.

## Results

*Effect of β-glucan and G-CSF on WBC count*. Treatment with CY induced significant myelosuppression in BDF-1 mice within four days as revealed by the decrease of leukocytes from 10.34±1.81 $\times 10^6$ to 1.47±0.59 $\times 10^6$ cells/ml (–86%) in the peripheral blood of control mice (Figure 1). In these mice, recovery of the WBC number occurred within seven days after myelosuppressive conditioning, followed by a transient compensatory overproduction of leukocytes. Treatment with β-glucan alone slightly elevated the WBC count in the late phase of hematopoietic recovery compared to control mice. In contrast, G-CSF as a single agent was able to diminish the strong decrease of leukocytes to some extent, although due to inter-individual variations, the WBC counts of G-CSF-treated and control mice were not significantly different, neither on day 1 ($p$=0.070) nor day 4 ($p$=0.072). Co-administration of

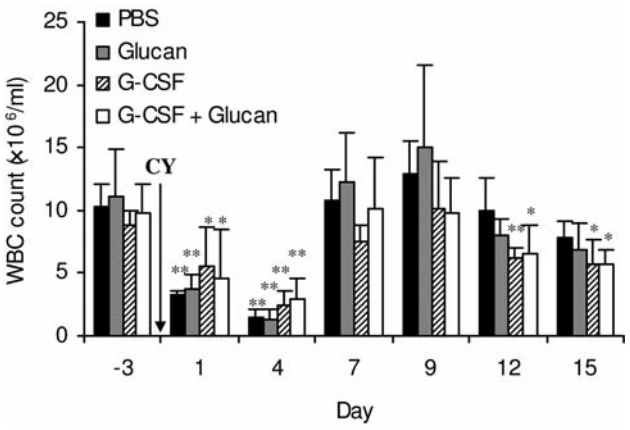


Figure 1. *Kinetics of the white blood cell (WBC) count in CY-treated leukopenic mice administered β-glucan and/or G-CSF. CY (200 mg/kg) was injected i.p. into BDF-1 mice on day 0. Subsequently, the mice received oral β-glucan (400 μg/mouse) and/or s.c. G-CSF (125 μg/kg) once daily from day 0 to day 5. Control mice received 0.1 ml PBS. The WBC number was determined at the indicated time points by automated cytometry. The data represent the mean±SD of eight mice per group (control: seven mice) from one experiment. Significant reductions of the WBC count within the groups between prior and after treatment with CY are indicated by *p<0.05 and **p<0.005.*

mice with both agents led to a slightly greater WBC number on day four after myelosuppressive treatment than that of administration of G-CSF alone.

*Effect of β-glucan and G-CSF on segmented granulocytes*. The number of segmented granulocytes remained stable in control mice one day after administration of CY before strongly declining until day four from 1.38±0.34 $\times 10^6$ to 0.07±0.06 $\times 10^6$ cells/ml (–95%) (Figure 2). β-Glucan failed to diminish the CY-mediated loss of segmented granulocytes, whereas G-CSF-treated mice were protected from strong leukopenia. Furthermore, treatment with G-CSF initially increased the number of segmented granulocytes significantly above the level found in control and β-glucan-treated mice. Mice receiving the combined treatment showed a slightly but not significantly higher number of segmented granulocytes than mice treated with G-CSF alone during the phase of highest myelosuppression. Subsequently, mice treated with β-glucan alone revealed a higher expansion of segmented granulocytes than mice receiving G-CSF only. Metamyelocytes, largely representing the juvenile granulocytes, showed a course comparable to that for segmented granulocytes (data not shown).

*Effect of β-glucan and G-CSF on monocytes*. The myelosuppressive treatment induced a rapid decrease of monocytes in the peripheral blood within one day, which was significantly mitigated only by G-CSF (Figure 3). However,

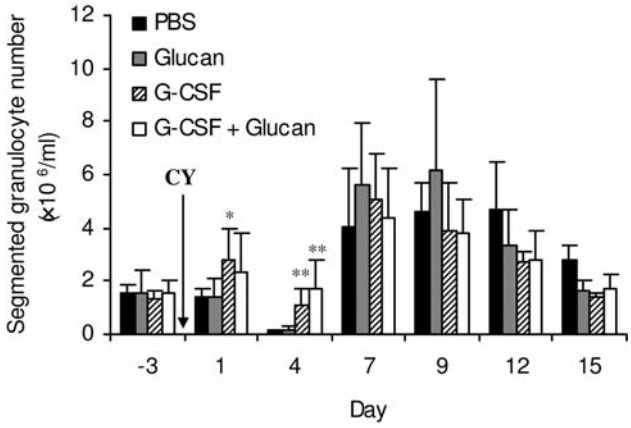


Figure 2. *Kinetics of segmented granulocyte numbers in CY-induced leukopenic mice administered β-glucan and/or G-CSF. CY (200 mg/kg) was injected i.p. into BDF-1 mice on day 0. Subsequently, the mice received oral β-glucan (400 μg/mouse) and/or s.c. G-CSF (125 μg/kg) once daily from day 0 to day 5. Control mice received 0.1 ml PBS. Segmented granulocytes were identified by characteristic morphology of their chromatin using a light microscope and quantified by manual counting of one representative field comprising 100 leukocytes on the slide. Data represent the mean±SD of eight mice per group (control: seven mice) from one experiment. Significant differences from the PBS-treated group are indicated by *p<0.05 and **p<0.005.*

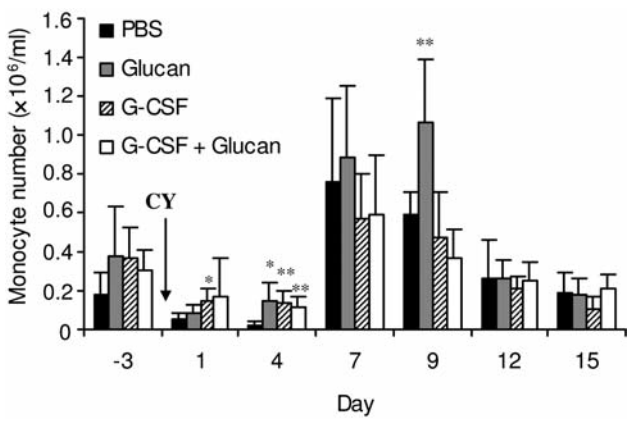


Figure 3. *Kinetics of monocyte numbers in CY-induced leukopenic mice administered β-glucan and/or G-CSF. Cy (200 mg/kg) was injected i.p. into BDF-1 mice on day 0. Subsequently, the mice received oral β-glucan (400 μg/mouse) and/or s.c. G-CSF (125 μg/kg) once daily from day 0 to day 5. Control mice received 0.1 ml PBS. Monocytes were identified by their characteristic morphology using light microscopy and quantified by manual counting of one representative field containing 100 leukocytes on the slide. The data represent the mean±SD of eight mice per group (control: seven mice) from one experiment. Significant differences from the PBS-treated group are indicated by *p<0.05 and **p<0.005.*

treatment with β-glucan started to significantly increase the monocyte number at day four after application of CY, compared with control mice. In addition, β-glucan-treated mice had a significantly higher number of monocytes at day nine compared with G-CSF-treated or control mice ($p<0.004$ and $p<0.001$, respectively). The combined treatment was not superior to the stimulatory effect of treatment with the single agents on monocyte recovery.

## Discussion

The effects of G-CSF and of particulate or soluble β-glucans on normal or injured murine bone marrow have been evaluated separately in several studies. In the present study, we compared the hematopoiesis-promoting effect of a soluble β-(1-3),(1-6)-D-glucan from *S. cerevisiae* with that of recombinant human G-CSF in myelosuppressed mice. Induction of strong leukopenia in the peripheral blood within four days and transient overproduction of WBCs during hematopoietic recovery was within the range described by others (9, 18, 22). Similar to studies utilizing the orally administered marine β-glucan Phycarine and the β-glucan SCCA derived from the mushroom *Sparassis crispa*, the β-glucan in this study failed to protect mice from the strongly reduced peripheral blood cellularity during the first six days after CY-mediated myelosuppression (9, 11). This could be due to the four-day period reportedly required for β-glucan uptake and transportation to the bone marrow by gastrointestinal macrophages (23). Within the marrow, the macrophages were demonstrated to process larger glucan molecules into smaller biologically active pieces which were then released (23). In the Phycarine study, the control mice showed suppressed WBC counts up to day 10 after treatment with CY. Therefore the fast recovery of the WBC count in control mice of our study could have hidden a potential stimulatory effect of β-glucan on hematopoiesis (11). The strong stimulatory effect of G-CSF on the bone marrow production of segmented granulocytes is in accordance with numerous studies of myelosupressed and healthy rodents, as well as in patients after chemotherapy or hematopoietic stem cell transplantation (1-5, 24). The peak appearance of segmented granulocytes in the peripheral blood after one day of treatment with G-CSF is caused by a manifold amplification of cell divisions during granulocytopoiesis and the rapid release of neutrophils into the circulation (24). The failure of G-CSF to significantly accelerate the recovery of the total WBC count could be due to the strong loss of lymphocytes (not shown), which represent one third of peripheral leukocytes and whose production is mostly unaffected by this growth factor. Subcutaneously injected G-CSF and *i.v.* injected β-glucan were described to synergize in the reconstitution of impaired murine hematopoiesis after irradiation (13). However, the slight elevation of segmented granulocytes after oral co-administration of β-glucan in this study may indicate a weak additive effect of both agents on

enhancing the proliferation and maturation of HPCs as similarly described from the mobilization of murine PBPCs (12). Additive activity could result from the reported β-glucan-mediated enhanced secretion of CSFs such as G-CSF or GM-CSF by bone marrow cells and leukocytes as subsequent mediators of hematopoietic activity (6, 8, 17-19). The relatively low to moderate amounts of GM-CSF or G-CSF released by β-glucan-activated splenocytes or human cord blood monocytes, however, may reflect the limited potential of these agents to promote hematopoiesis when delivered orally (11, 18, 19). Recovery of monocytes in the peripheral blood was shown to be enhanced after *i.p.* administration of β-glucans in healthy or myelosuppressed mice and following oral administration in cancer patients (6, 7, 20, 25). The beneficial effect was assigned to the induced production and release of monocyte growth factors by bone marrow and leukocytes leading to enhanced proliferation and maturation of monocytic precursors (6, 8, 18, 25). The later onset of the stimulatory effect of β-glucan on monocyte recovery as compared to G-CSF supports the notion of the induction of hematopoietic growth factors, although granulopoiesis was not enhanced. The particularly augmented production of monocytes could be facilitated by the reported high expression of dectin-1 and CR3 on myeloid progenitor cells of the bone marrow (14, 15). In addition, treatment of mice with CY is known to further up-regulate expression of dectin-1 and CR3 on leukocytes (18). The stimulatory effect of G-CSF on monocytopoiesis observed in this study and shown by several other animal and patient studies in the past remains mechanistically unclear, since direct activation of monocyte progenitors with G-CSF has not yet been demonstrated (3, 24). In conclusion, we demonstrate that an orally administered soluble β-(1-3),(1-6)-D-glucan accelerates the recovery of monocytes in the peripheral blood of leukopenic mice, while the carbohydrate compound alone has no visible effect on granulopoiesis, but appears to be weakly additive when used in combination with G-CSF.

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