CVI Accepts, published online ahead of print on 8 November 2006
Clin. Vaccine Immunol. doi:10.1128/CVI.00284-06

Enhancement of umbilical cord blood cell hematopoiesis by Maitake beta glucan is mediated by G-CSF production

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Source of support: the U.S. Army Medical Research and Materiel Command under DAMD 17-01-1-0323, NIH NCI 29502, NIH NCAM 1-P50-AT02779 and the Children's Blood Foundation

Running Title:

Maitake beta-glucan enhances umbilical cord blood cell hematopoiesis *in vitro*

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Abstract:

Maitake beta glucan (MBG) is an extract from the fruit body of the *Grifola frondosa* mushroom that is being widely used to treat cancer in Asia. We have previously reported that MBG enhanced mouse bone marrow cell (BMC) hematopoiesis *in vitro* and protected BMC from Doxorubicin (DOX) toxicity. In the current study, we investigated the ability of MBG to enhance hematopoiesis and to reduce the toxic effects of DOX on fresh human umbilical cord blood (CB) cells. MBG treatment significantly enhanced the CFU-GM response over the whole dose range 12.5-100ug/ml ($p<0.05$). The addition of MBG to DOX treated CB cells significantly protected CFU-GM colony formation from DOX toxicity which otherwise produced strong hematopoietic repression. MBG also partially replaced rhG-CSF as shown by significant augmentation of the CFU-GM response in the absence of rhG-CSF. We found that MBG induces G-CSF production in CB $CD33^+$ monocytes as detected by intracellular cytokine flow cytometric assessment. In contrast we found that adult peripheral blood monocytes did not produce a significant G-CSF response to MBG whereas both adult and CB monocytes produced G-CSF in response to LPS. These studies comprise the first evidence that MBG induces hematopoietic stem cell proliferation and differentiation of CFU-GM in umbilical CB cells and acts directly to induce G-CSF.

Introduction:

Maitake beta-glucan (MBG) is an extract from the fruit body of the *Grifola frondosa* mushroom that is widely used in Asia for the treatment of cancers, although the mechanism(s) of action are unclear (4). MBG contains glucan polysaccharide compounds that have a beta-1,6 glucopyranoside main chain with branches of beta-1,3 linked glucose (24). Experimental animal studies suggest that MBG administered orally activates the host anti tumor response through effects on the immune system rather than by direct cytostatic or cytotoxic effects on tumor cells (19, 37). However, dose response relationships have not been shown (15, 21). Since other beta glucans have been found to reduce myelosuppression and to enhance hematopoiesis *in vitro* and mobilization of stem cells *in vivo* in animal models (16, 27, 33), we initially tested the effect of MBG on mouse bone marrow cells. These studies showed for the first time that MBG enhanced murine bone marrow cell proliferation and differentiation into CFU-GM in a dose dependent manner(22). In the presence of chemotherapy drug Doxorubicin (DOX), MBG promoted bone

marrow cell viability and protected bone marrow stem cell CFU-GM from DOX induced hematopoietic toxicity. Based on our studies in the mouse we are interested in the possible use of MBG in myelosuppression secondary to cancer chemotherapy and for *ex vivo* expansion of CB cells prior to stem cell transplantation. CB is increasingly used for stem cell transplantation for leukemia and CB progenitor cells have distinctive immunological properties that enable *ex vivo* expansion (2, 3). The aims were to assess potential ability of MBG to enhance umbilical CB hematopoietic progenitor cell activity in a CFU-GM assay and to determine the mechanism of action. As reported here, we found that MBG enhanced human umbilical cord blood cell proliferation and differentiation into CFU-GM *in vitro*. MBG also protected CB cell colony forming potential from the damaging effects of DOX toxicity. In the absence of G-CSF, MBG enhanced the colony forming ability of CB cells over control cultures. Examination of the effects of MBG on cytokine response showed that MBG induced intracellular G-CSF production in $CD33^+$ monocytes and that G-CSF was secreted. The studies show that MBG has specific dose related effects on human hematopoietic progenitor cells indicating potential for therapeutic use in myelosuppression. In addition the results support the possibility that MBG could enhance expansion of CB cells *ex vivo* to improve engraftment.

Material and Methods:

Chemicals and Reagents:

Maitake mushroom beta-glucan (MBG) is an extract from fruit body of Maitake mushroom (*Grifola frondosa*), which was made under patented methods (Japan Pat. No.2859843/US Pat. No.5,854, 404) and provided by Yuikiguni Maitake Corp. through the Tradeworks group. The extract was stored in a refrigerator at 4 ^{o}C under dark conditions until use. The lot of MBG used in this study was sent to NAMSA to test for endotoxin contamination using limulus amebocyte lysate (LAL) assay. The result showed that there was no detectable endotoxin activity (maximum level = 0.012 EU/ mg). MBG powder dissolved readily in RPMI 1640 with 25mM HEPES buffer and was initially prepared at a concentration of 20mg/ml and sterilized by filtration through 0.2 um cellulose acetate low protein binding membrane, and stored at –20^{o}C. The stock solution was diluted to the required concentration in RPMI 1640 medium freshly at the time of use.

Adriamycin PFS (Doxorubicin Hydrochloride, DOX), MW= 580; purity>98% (Pharmacia Inc) was diluted in RPMI 1640. Recombinant human G-CSF (rhG-CSF) and recombinant human IL-3

(rhIL-3) were obtained from Amgen (Thousand Oaks, CA) and Intergen (Purchase, NY), respectively.

Human Umbilical Cord Blood:

Human umbilical cord blood samples from healthy full term infants were obtained at the time of delivery and transferred into heparinized sterile blood collection tubes and studied freshly. Peripheral blood samples from healthy adults were obtained by venipuncture and studied freshly. This was an IRB approved study at Cornell University Weill Medical College.

Preparation of umbilical cord blood cells:

To prepare human umbilical cord blood cells for colony formation assay, red blood cells were removed by lysis with ammonium chloride buffer containing 155mM NH_4Cl, 0.1mM EDTA, 10mM $KHCO_3$, pH 7.4-7.6. Buffered ammonium chloride solution was added to human umbilical cord blood at 10:1 (v/v). The cell suspension was vortexed gently and placed on ice for 20 minutes to allow the red blood cells to lyse. The cells were washed twice with Iscove's Modified Dulbecco's Medium (IMDM) and then resuspended in IMDM. The mononuclear cells were stained with trypan blue, counted by light microscope using a hemocytometer, and the concentration of viable mononuclear cells was adjusted to $5X10^4$ cells/ml.

Colony Formation Unit assay (CFU)

The colony-formation assay was carried out under defined conditions by standard techniques (StemCell Technologies Inc. Vancouver, Canada) (22). Briefly, a complete culture medium was prepared by adding growth factors, rhG-CSF and rhIL-3, and IMDM into a premixed methylcellulose culture medium (Methocult^{TM} H4230, StemCell Technologies Inc.; Vancouver, Canada), which contained methylcellulose, fetal bovine serum (FBS) and bovine serum albumin (BSA) in IMDM. The final adjusted concentrations were 1% methylcellulose, 30% FBS, 1% BSA, 10^{-4} M 2-mercaptoethanol and 2 mM L-glutamine, rh IL-3 10ng/ml, rhG-CSF 500ng/ml. For studies on whether MBG could replace growth factors, either rhG-CSF or rhIL-3 was omitted. Aliquots of 0.3 ml of the cord blood cell suspension ($5x10^4$cells/ml) were added to each tube in 2.7ml complete culture methylcellulose IMDM medium with addition of MBG or DOX at the required concentration in a volume of 20 ul. The tubes were then vortexed, and the mixtures were plated in duplicate into sterile 35x10mm Cell-Culture-Dishes (Fisher Scientific

International) at 1.1 ml /dish. Then all cultures were incubated in a water-saturated incubator at 37°C, containing 5% CO_2. After 14 days incubation, CFU-GM colonies consisting of 20 or more cells were identified and scored using an inverted microscope.

Intracellular cytokine assay

Intracellular cytokine production was assessed according to standard techniques using fresh cord blood or whole blood from normal adult donors. Blood was collected into sodium heparin vacutainer tubes and maintained at room temperature. Cord blood or adult peripheral blood was diluted 1:2 with RPMI 1640 containing 25mM HEPES buffer, 23ug glutamine per ml, 40 IU penicillin, and 40ug streptomycin per ml. Lipopolysaccharide (LPS) at 1ug/ml, or MBG at 100ug/ml, or medium as a control were added in the presence of Brefeldin A (BFA), Sigma, St. Louis, MO) at final concentration 10 ug/ml. After incubation in water-saturated incubator at 37°Cwith 5% CO_2, cells were removed from culture and initially stained with monoclonal antibodies directed against lineage specific surface antigens. Anti-human CD33 monoclonal antibodies (Mabs) used for cell surface staining was obtained directly conjugated to fluorescein isothiocyanate (FITC), (BD Biosciences, San Jose, CA). Cells were then permeabilized with FACS permeabilizing solution and then incubated with monoclonal antibodies directed towards the cytokines of interest. Monoclonal antibodies used for the intracellular detection of cytokine were obtained from BD Biosciences and R&D Systems, including: anti-GM-CSF PE (BD Bioscience, San Jose, CA), anti-GCSF PE (R&D System, Minneapolis, MN). After a final wash, the cells were resuspended in PBS and analyzed immediately by FACSCalibur flow cytometer (BD) using the Cell Quest software. Three hundred thousand total events were collected. Gating was established using forward and side light scatter and fluorescence back-gating with CD33 to define the populations. Data were analyzed by histogram overlay of activated and nonactivated cells.

Enzyme-linked immunosorbant assay (ELISA) to measure G-CSF

To test that intracellular cytokine production detected by intracellular assay tracked with secretion, parallel cultures were established as described above but carried out in the absence of BFA. Blood was incubated with LPS at 1ug/ml, or MBG at 100ug/ml for 18 hrs. After incubation, supernatants were collected by centrifugation at 1500rpm for 10 min. G-CSF levels in the supernatant were measured using the Quantikine HS G-CSF immunoassay, obtained from

R&D System (Minneapolis, MN). Briefly, 100ul of supernatant or each of the dilutions of the standard recombinant G-CSF was dispensed in duplicate into 96-well plates pre-coated with an anti-GCSF antibody. The plates were incubated at room temperature for 3hr. After washing, assessment of G-CSF was obtained by sandwich technique with a polyclonal antibody against G-CSF, which was conjugated to alkaline phosphatase. A substrate solution and amplifier solution, and stop solution were added sequentially. The plates were read using an ELISA plate reader at 490nm, λ correction 690nm within 30min. This ELISA system specifically recognizes human G-CSF, in the range of 1.25-80pg/ml.

Statistical analysis and data presentation:

Data are presented as mean percentage ± SD or mean ± SD.. CFU-GM from duplicate cultures were averaged and collected to compile the group data. As each cord blood came from a genetically distinct host we also normalized data from each separate cord blood sample by using the average of duplicate control cultures as 100% and then comparing the average of duplicate experimental cultures from each subject to this standard. Data were then compiled from the collected individual results to form the group results. Statistical analysis was performed using oneway ANOVA and multiple comparisons with SPSS version 12.01.

Results:

Effect of MBG on CFU-GM formation of umbilical cord blood cells:

Healthy full term infants' umbilical cord blood samples were collected after delivery. Red blood cells were removed by ammonium chloride lysis and mononuclear cells were counted by hemocytometer. Viability was determined by trypan dye exclusion and exceeded 90% on all samples. To study the effects of MBG on cord blood cell hematopoietic activity, cord blood cells from 8 neonates were cultured at a final concentration of $5X10^3$ per dish in duplicate in the presence or absence of multiple concentrations of MBG in the methylcellulose complete culture medium. The actual number of colonies in these cultures ranged from 38 to 97 per plate in individual experiments. Duplicates were within10-15% of each other. To normalize the genetic distinct of infant's cord blood sample, for each CB sample, mean colony counts in control cultures with no MBG were considered as 100% response and used to calculate the percentage of the difference from control CFU-GM. As shown in Fig.1, MBG enhanced the colony forming

ability of cord blood cells in the group when MBG was added at doses ranging from 12.5 to 100 ug/ml. Differences were significantly greater than those of control cultures in the group when MBG was present at 12.5ug/ml ($p= 0.015$), at 25ug/ml $p= 0.003$, at 50ug/ml ($p<0.001$), and at100ug/ml ($p<0.001$). Addition of MBG at 100ug/ml increased the colony counts up to 140% of controls. When we evaluated this difference using the actual mean number of CFU-GM colonies, we obtained essentially the same results (data not shown).

When MBG was added at 200ug/ml, a slight but not significant increase was seen when compared to cultures without addition of MBG. This was not due to any cytostatic or cytotoxic effects. Adding MBG at 200 ug/ml showing no cytotoxic effect on cord blood cells' viability, which was tested by the XTT cytotoxicity assay (data are not shown). As also shown in our previous murine studies(22).

Protective effect of MBG on cord blood CFU-GM formation in the presence of DOX:

Experiments were then carried out to determine whether MBG was capable of protecting the proliferation and differentiation of progenitor CFU-GM in cord blood cell samples from hematopoietic toxicity caused by DOX treatment. According to our previous report (22) and that of others (34) we used an appropriate dose range of doxorubicin from 7.5-120ng/ml to detect suppressive effects. After removing red blood cells, cord blood cells from 4 infants were cultured as before in methylcellulose medium containing rhIL-3 10ng/ml and rhG-CSF 500ng/ml, in the presence of serial dilutions of DOX (7.5-120ng/ml), with or without MBG at 100ug/ml. As shown in Fig. 2A, in the presence of low dose (7.5 ng/ml) DOX, MBG was still able to significantly enhance the CFU-GM response of CB cells over control cultures with no DOX present. The dose of MBG that elicited the maximum effect varied somewhat among individual cord blood samples, but over all was greatest at doses of 50 ug/ml ($p=0.018$) or 100 ug/ml ($p=0.003$), MBG was significantly protective against the toxicity of DOX for each CB sample. With high dose DOX (120ng/ml), MBG enhancement fell to 33.3% of control cultures. However in the absence of MBG, 120ng/ml DOX treatment alone decreased CFU-GM response to a nadir of 16% of control. Thus although the enhancing effect of the optimal concentration of MBG (100 ug/ml) on CFU-GM response declined with increasing doses of DOX, the CFU-GM responses in the presence of MBG remained significantly higher than with DOX alone across the entire range of DOX ($P=0.001$). When we calculated the CFU-GM response as percentage of

control using CFU-GM responses to MBG 100ug per ml as the 0 control, we found essentially similar levels of protection and significance, as shown in Fig. 2B.

Effect of MBG on CFU-GM formation in the absence of rhG-CSF:

Since in the previous experiments, MBG appeared to induce CFU-GM, we next sought to determine if this effect were dependent upon the presence of G-CSF. To test this, 4 cord blood samples were collected and cultured in methylcellulose medium containing rhIL-3 10ng/ml, but in the absence of rhG-CSF. The effect of varying doses of MBG was assessed in comparison with control cultures that were supplemented with both rhIL-3 (10ng/ml) and rhG-CSF (500ng/ml). As shown in Fig.3, in the absence of rhG-CSF, colony counts were reduced to 51.6% of controls, while in the presence of MBG at the dose range of 25ug/ml to 100ug/ml, the CFU-GM response exceeded 80% of controls. The results showed that statistically equivalent levels of CFU-GM activity were observed when MBG was present compared to controls cultured in medium with rhG-CSF. We separately carried out titrational studies and determined that the optimal concentration of rhG-CSF for CFU-GM activity is 250 to 500 ng/ml. In contrast, MBG could not substitute for rh IL-3. In the absence of rhIL-3 with only rhG-CSF present, the colony counts decreased to 41.6% of controls, and addition of MBG over the same range of dose levels did not improve the CFU-GM response (data not shown).

MBG induction of intracellular G-CSF in CD33$^+$ cord blood cells

Experiments were undertaken to determine whether MBG induced granulocyte or granulocyte-macrophage stimulating growth factors in cord blood. Umbilical CB samples from 14 healthy full term infants were collected and studied. Mononuclear cells were cultured with MBG for 4 hrs or 18hrs in the presence of BFA, which disrupts the structure and function of Golgi apparatus, inhibiting intracellular transport so that cytokines produced during the culture period will not be secreted. After 4hrs of incubation, as shown in Fig.4, intracellular production of G-CSF in CD33$^+$ cord blood cells was significantly higher than in cultures without MBG ($p=0.04$). At 4hrs LPS did not show a significant effect on cord blood G-CSF production. To determine if this effect was specific to CB monocytes, we also obtained fresh peripheral blood samples from 6 adults. As shown in Fig.5, after18hrs of culture, about 20% of cord blood monocytes were producing G-CSF compared to 10% of monocytes in parallel control cultures ($p=0.04$). In contrast induction of G-CSF in monocytes in the adult group at 18hrs was not different compared

to unstimulated cultures, although a few adults showed a slight response. As shown in Fig.5, in contrast to results at 4hrs, the effect of LPS on cord blood G-CSF response was stronger than that of MBG at 18hrs. CB monocyte response to LPS was higher compared to that of adult monocytes (p= 0.04). MBG did not induce intracellular GM-CSF production in $CD33^+$ cord blood cells although LPS did significantly induce GM-CSF production (data not shown).

MBG induces secretion of G-CSF by cord blood $CD33^+$ cells

To determine whether intracellular cytokine detection was associated with secretion, we used a highly sensitive ELISA assay for G-CSF detection. Seven healthy full term infants' umbilical cord blood were collected after delivery. Five healthy adults' peripheral blood samples were obtained from healthy volunteers. Supernatants from cultures of cord blood cells and peripheral blood cells were collected after culture with MBG or LPS and tested. As shown in Fig.6, MBG induced a significant increase in G-CSF secretion from CB cells compared to control unstimulated cultures. In contrast MBG did not induce secretion of G-CSF from adult peripheral blood mononuclear cells. Secretion levels of G-CSF in response to LPS were comparable between CB cells and adult peripheral blood cells.

Discussion:

The studies described here are the first to show that MBG induces the proliferation and differentiation of CFU-GM colonies from human umbilical cord blood progenitor cells *in vitro* and to demonstrate that MBG stimulates the intracellular production and secretion of G-CSF in cord blood $CD33^+$ monocytes. Previously we found that MBG stimulated hematopoiesis in murine bone marrow cells and enhanced recovery of the CFU-GM response from DOX induced hematopoietic suppression (22). In the present investigation we found a similar dose range suggesting a fundamental similarity between mouse and human progenitor cell CFU-GM response to MBG *in vitro*. As observed with murine bone marrow cells, addition of MBG above the optimal range at 200 ug/ml showed only a modest effect on colony forming ability of CB cells. This was not due to cytostatic or cytotoxic effects. Although a related Maitake beta glucan preparation, Grifron-D, has been reported to have cytoxtoxic effects on PC-3 human prostate cells at a dose level of 480ug/ml or greater (13), we did not find this effect using various tumor cell lines including PC-3 with the MBG extract described here (HL, unpublished data). Other

studies of the anti- tumor effects of Maitake mushroom in the mouse appears to indicate involvement of the host immune system (1, 15) rather than direct anti-tumor effects.

MBG is one of the fractions of extract from the fruit body of *Grifola frondosa*, which has shown the strongest anti-tumor effect (1, 17, 25). The MBG used in these studies was prepared under standardized conditions, has a molecular weight of 1,000,000, is highly soluble, and does not contain endotoxin. Membrane receptors that mediate beta glucan response include the murine Dectin-1 receptor, (5, 6) the human beta GR receptor (40, 41), CR3 (39), the scavenger receptors(35), and lactsylceramide(43). Activation of both Dectin-1 and Beta GR may lead to inflammatory cytokine production (10, 11). Dectin-1 expression has been shown to enhance TLR-mediated activation of nuclear factor kappa B by beta-glucan-containing particles. In macrophages and dendritic cells, Dectin-1 and TLRs are synergistic for the production of interleukin 12 and tumor necrosis factor alpha (14). For orally active glucans mechanism of beta glucan action has been shown to involve monocyte degradation of glucan into smaller fragments in the bone marrow prior to uptake by effector cells (18). A soluble barley CR3-specific beta-glucan has been shown to induce a primed state of CR3 that triggered killing of iC3b-target cells that were otherwise resistant to cytotoxicity (36) to enhance anti tumor immunity (8, 23).

Other glucans have been previously shown to enhance hematopoiesis *in vitro* and mobilization of hematopoietic cells *in vivo.* Using a partially purified glucan derived from zymosan, the first report showed that injection caused increased CFU-GM after 10 days in the spleen and to a much lesser extent in the bone marrow (7). Patchen and Lotzova studied glucan from the yeast *Saccharomyces cerevisiase* and reported that glucan mobilized progenitor cells to the spleen and periphery (28), increasing hematopoiesis in the spleen and increasing pluripotent stem cell activity in the bone marrow (30). The effects on splenic hematopoiesis were macrophage dependent while the mobilization effect was not (29). Intravenously administered glucan was also found to improve survival of sublethally irradiated recipient mice when given one day before or after radiotherapy (31), and the effect of giving both glucan and G-CSF was synergistic (32). Recent studies show that after hematopoietic injury or G-CSF mobilization, stroma cells express iC3b and tether $CR3^+$ hematopoietic progenitor cells (HPCs) suggesting that beta glucan may be able to enhance their proliferation (9). PGG-Glucan has been shown to

310 enhancing the effect of G-CSF on hematopoiesis in the C3H/HeN mouse(27), and in human
311 bone marrow cells (38). However PGG-Glucan does not induce G-CSF production.
312
313 In the present study we show that MBG has significant hematopoietic potential to promote the
314 proliferation and differentiation of progenitor stem cells in cord blood. MBG has been shown to
315 activate the production of G-CSF in CB CD33+ monocytes for the first time. Early induction of
316 G-CSF in cord blood by MBG in CB monocytes appears to be the mechanism of CFU-GM
317 enhancement and may reflect the unique hematopoietic potential of this compartment. Further
318 investigation of enhancement of MBG on other lines such as CFU-MK, BFU-E, LTI-IC, as well
319 as the signaling pathway involved in the induction of G-CSF elicited by MBG may reveal
320 fundamental information concerning the regulation of the accessory cord blood cells important
321 for *ex vivo* expansion of CB progenitor and pluripotent stem cells for transplantation and the role
322 of beta glucan in the enhancement of hematopoiesis.

Figure Legends

Fig.1. Effect of MBG on CFU-GM formation of umbilical cord blood cells. Cord blood cells from 8 healthy infants were prepared by ammonium chloride lysis of red cells and treated with MBG at different doses as indicated, and then cultured in methylcellulose medium for 14 days, using cultures without MBG as controls. Data are presented as mean ± SD of percentage of colony counts scored in control cultures without MBG, *p<0.05, **p<0.001 vs. controls.

Fig.2. Protective effect of MBG on CFU-GM formation of cord blood cells in the presence of DOX. Cord blood cells were prepared as described in Figure 1, and treated with serial doses of DOX in the absence or presence of MBG (100 ug/ml) and cultured in methylcellulose medium for 14 days. (A.) Data are presented as mean ± SD of percentage of colony counts scored in control dishes (which did not receive drug). Data represents the combined results of four separate experiments. * p<0.05 vs. DOX alone. (B.) Data are presented as mean ± SD of percentage of colony counts scored in dishes with MBG 100ug/ml alone with no DOX. *P<0.05 vs. MBG alone.

Fig.3. Effect of MBG on CFU-GM formation in the absence of rh G-CSF. Cord blood cells were prepared as described in Figure 1, treated with MBG at different doses as indicated, and then were cultured in the absence of rh G-CSF in methylcellulose medium for 14 days. Results were compared to control cultures without MBG but with rh G-CSF 500 ng/ml and rh IL-3 10ng/ml. Data show mean ± SD of percentage of colony counts scored in control dishes (which was in the presence of both rh G-CSF and rh IL-3). Data represents the combined results from 4 different umbilical cord blood samples.

Fig.4. MBG induction of intracellular G-CSF in $CD33^+$ cord blood cells. Umbilical cord blood was incubated with MBG 100ug/ml or LPS at 1ug/ml for 4 hours. Intracellular cytokine assays were carried out as described in Methods with anti-CD33 FITC for cell surface staining and anti-GCSF monoclonal antibody to detect intracellular G-CSF. Data present the combined results of umbilical cord blood samples from 5 healthy term infants. *p<0.05 vs. controls with no stimuli.

Fig.5. MBG stimulates intracellular G-CSF production in $CD33^+$ cells of CB but not adult peripheral blood. Umbilical cord bloods or peripheral bloods were incubated with MBG at 100ug/ml or LPS 1ug/ml for 18hrs. Then the intracellular cytokine assay was carried out as described in Methods, using anti-CD33 FITC for cell surface staining. After treated with permeabilizing solution, anti-GCSF antibodies were added to detect intracellular production of G-CSF. Data show means ± SD of the combined results of 9 cord blood samples from healthy term infants, and 6 peripheral blood samples from healthy adult donors. For cord blood, MBG group vs. controls P=0.006*, LPS group vs. controls P=0.005*, MBG group vs. LPS group P=0.137. For adults' peripheral blood, MBG group vs. controls P=0.44, LPS group vs. controls P=0.04*.

Fig.6. MBG induces secretion of G-CSF by cord blood cells. Umbilical cord blood or peripheral blood was incubated with MBG 100ug/ml or LPS 1ug/ml for 18hrs, and supernatants were collected. Secretion of G-CSF in the supernatant was detected with HS human G-CSF ELISA. Data show means ± SD of the combined results of cord blood from 7 healthy infants or adult peripheral blood from 5 healthy donors. For cord blood, MBG group vs. controls with no stimuli P=0.02*, LPS group vs. controls P<0.001**, MBG group vs. LPS group P=0.11. For adults' peripheral blood, MBG group vs. controls P=0.791, LPS group vs. controls P<0.001**, MBG group vs. LPS group P<0.001**.

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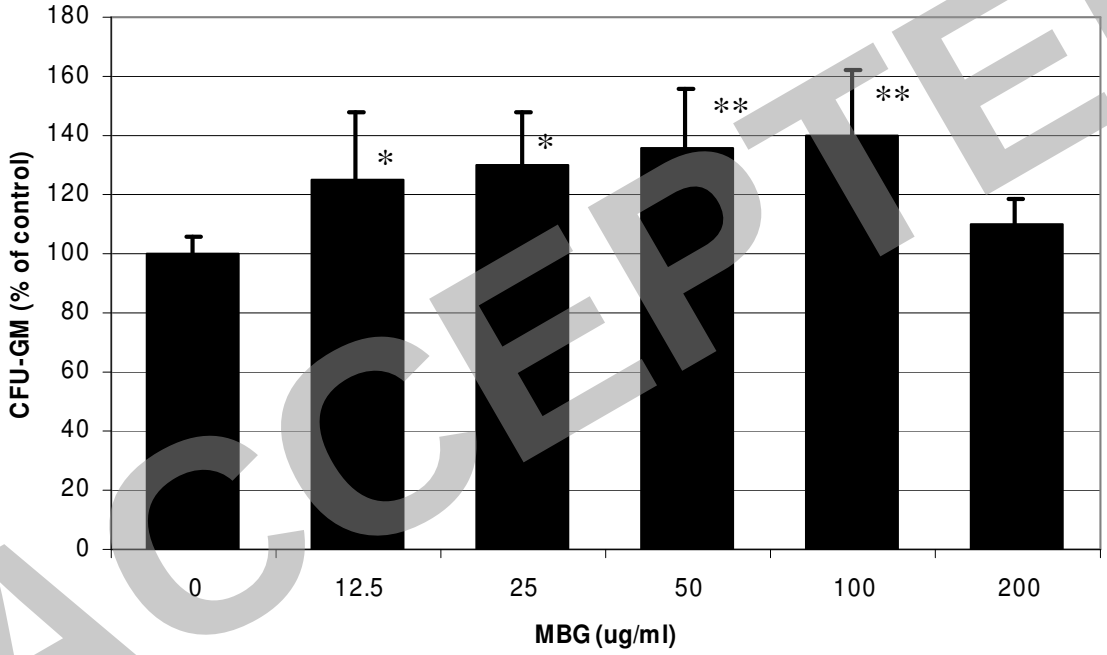


Figure 1: Lin et al.

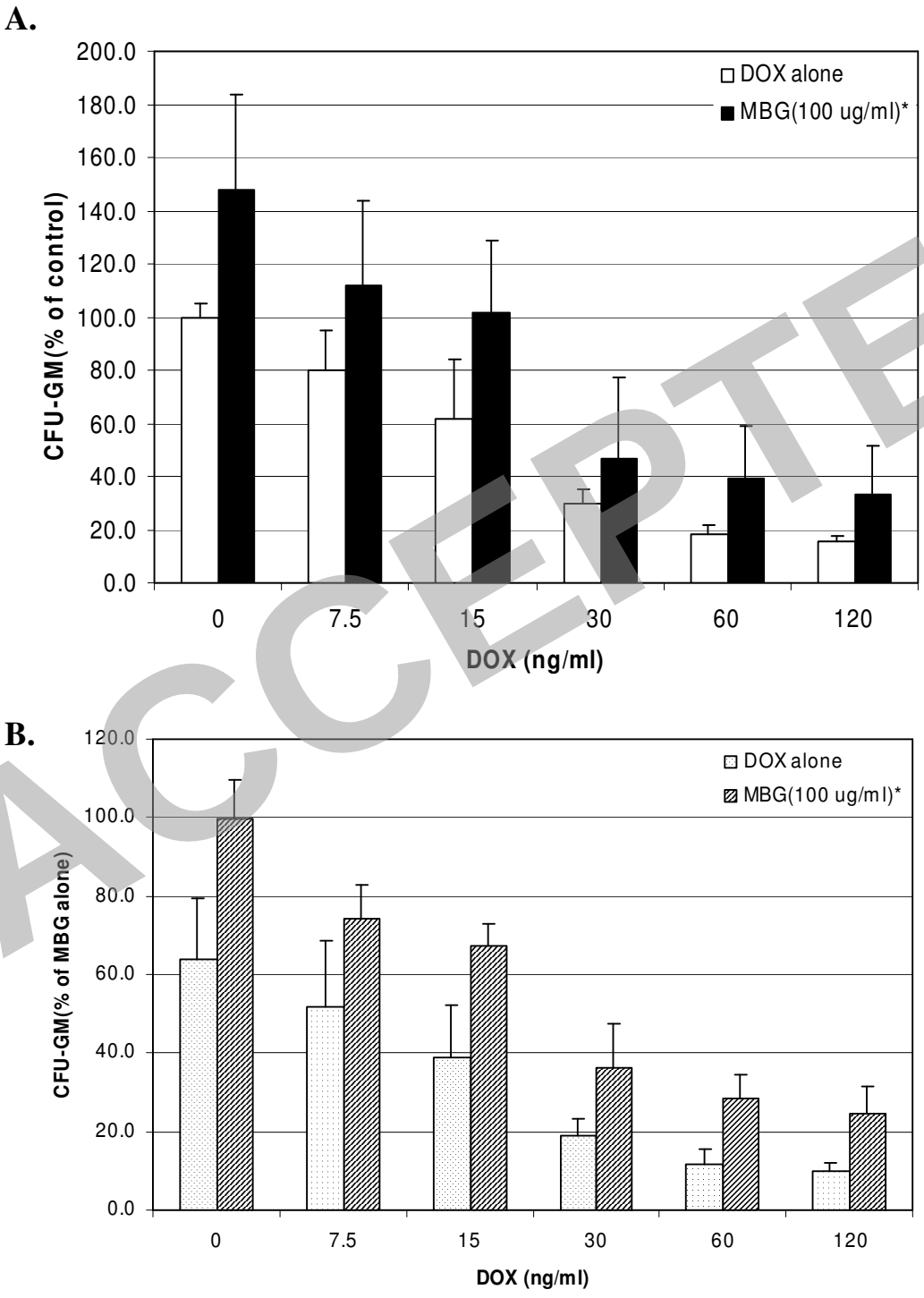
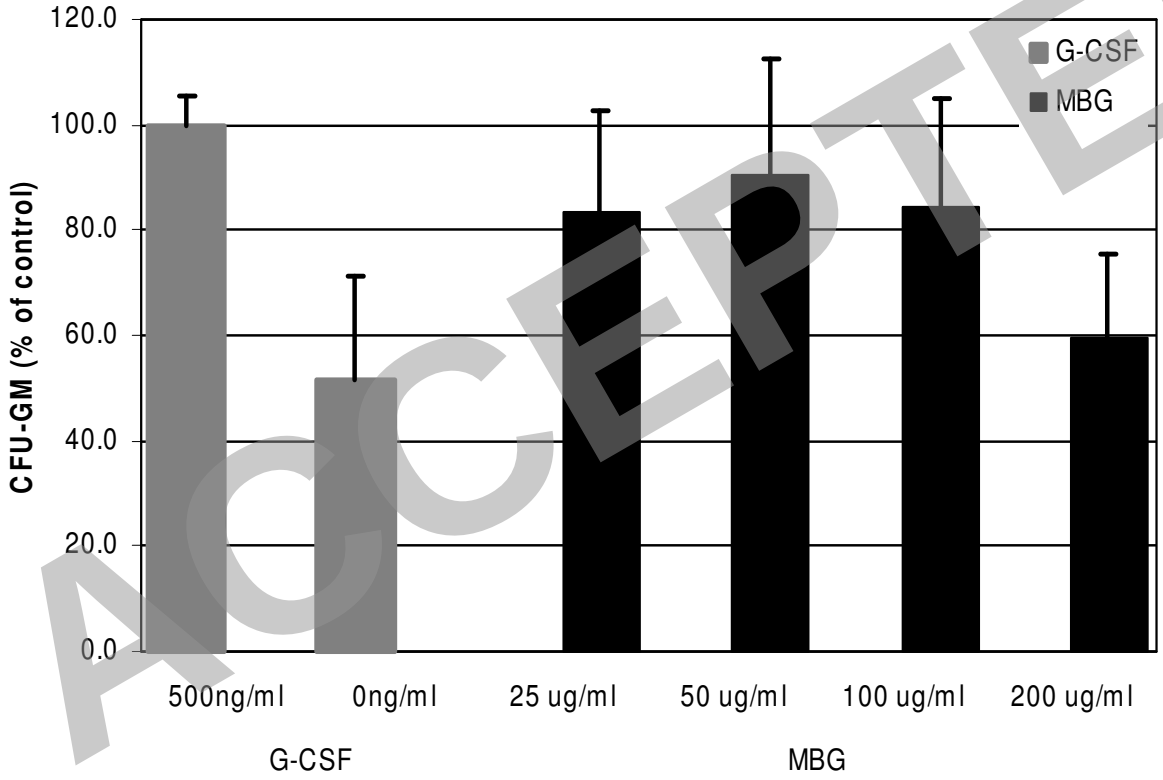


Figure 2: Lin et al.



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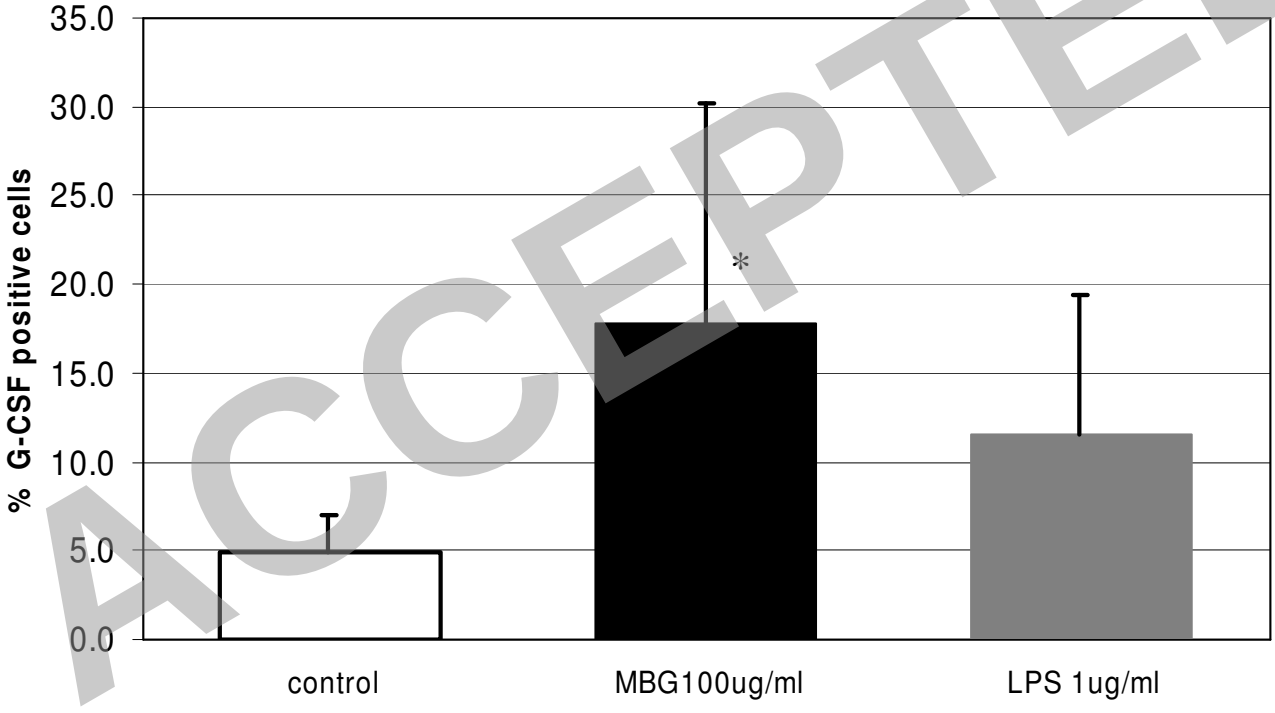

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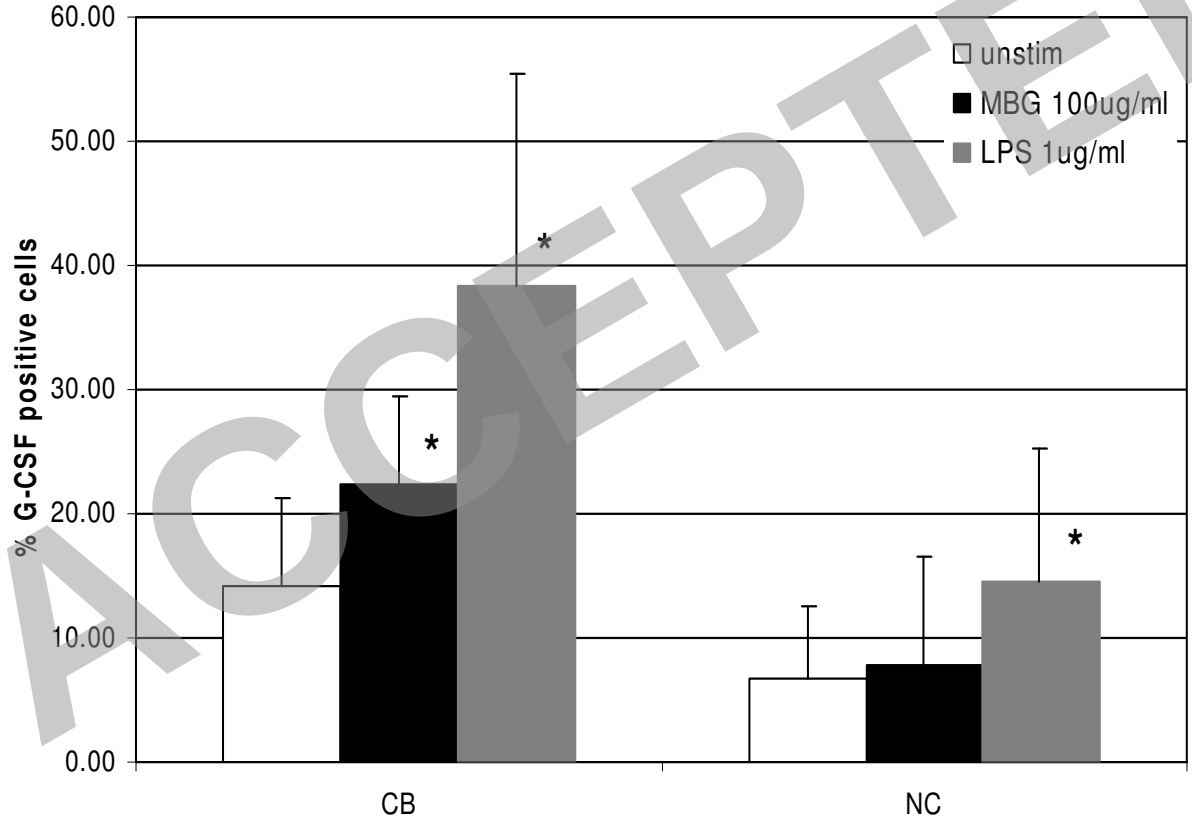

Figure 5: Lin et al.

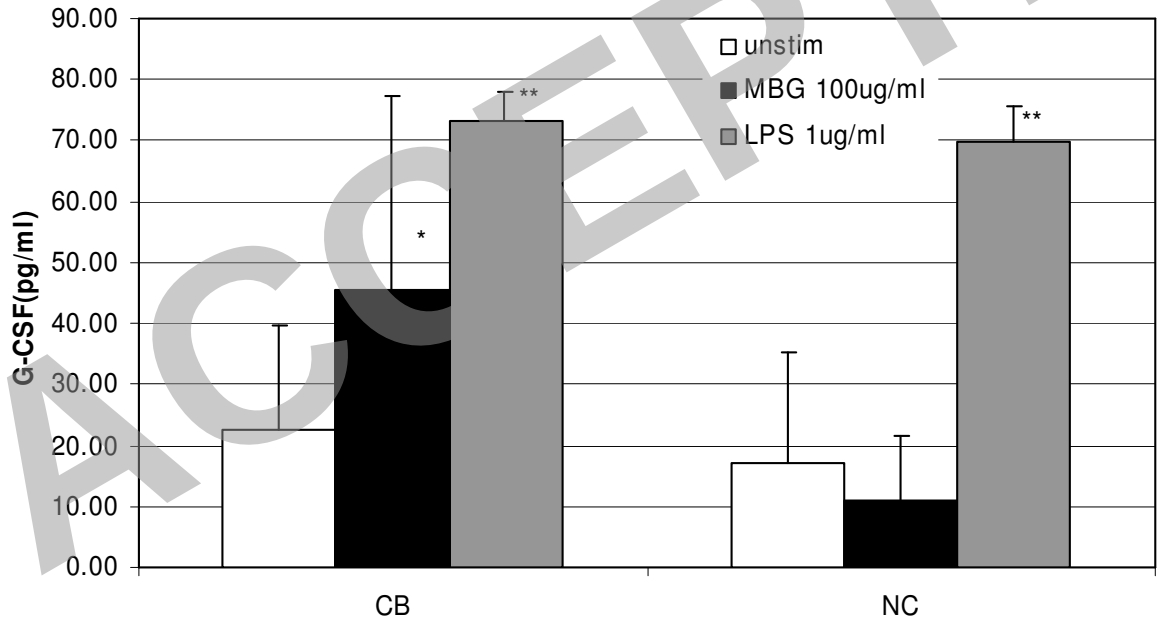

Figure 6:Lin et al