Effect of Human Pregnancy-Specific β1-Glycoprotein on Blood Cell Regeneration After Bone Marrow Transplantation (44225)

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Abstract. **Pregnancy-specific β1-glycoprotein (PSG) is composed of a family of highly homologous proteins initially isolated from human placenta and pregnancy serum. Recent studies showed that PSGs are also present in a number of ectopic sites, including uncultured peripheral blood and bone marrow cells. This report aims at studying the *in vivo* effect of the PSGs on murine hematopoiesis. The profile of recovery of blood cells after transplantation of viable nucleated bone marrow cells in γ-irradiated mice with and without the administration of the purified human protein was studied. Five groups of mice were given 0.1 μg human serum albumin, 0.1 μg IL6, 1 μg PSG, 10 μg PSG, and 50 μg PSG, respectively, per mouse per day consecutively for 20 days. The mice were bled once every 2 days, and the platelet and WBC counts were determined using a Nebauer hemacytometer (Hausser Scientific, Buffalo, NY). The recovery of platelet count after bone marrow transplant was much faster in mice receiving 1 μg PSG/day than in animals in any other group. On Day 20 post-transplant, the platelet count of animals in this group reached 178,600 ± 15,759/μl (mean ± standard deviation) which was significantly ($P < 0.05$) higher than that of any other group. On Day 26, the platelet count reached a low normal value of 190,844 ± 6,380/μl with a range of 185,420–200,500/μl. This value was 3-fold higher than that of the control group (68,600 ± 15,486/μl in the human serum albumin group). Mice given 1 μg or 10 μg PSG/day also had their WBC count recover significantly faster and achieved a normal value (12,440 ± 3,680/μl for the 1-μg PSG group, and 12,154 ± 3,016/μl for the 10-μg PSG group) within the experimental period. On the other hand, the controls, or mice given 50 μg PSG/day did not recover as rapidly and did not achieve a normal WBC count within the experimental period. These results suggest that human placental PSGs enhance platelet and WBC recovery after bone marrow transplant.**

[P.S.E.B.M. 1998, Vol 217]

Placenta is a rich source of hematopoietic growth factors including granulocyte-macrophage colony stimulating factor, eosinophil colony stimulating factor, interleukin-2 (IL2), interleukin-6 (IL6), etc., (1–3), and the pregnancy-specific β1-glycoproteins (PSGs) (4). When first identified, PSGs were believed to be synthesized only by the placental syncytiotrophoblast. Subsequent studies showed that PSGs were also synthesized by a number of nonplacental tissues (reviewed in Ref. 5). The PSG family is composed of members encoded by 11 highly homologous genes clustered on the long arm of chromosome 19 (6). These genes give rise to a number of differentially spliced products (5, 7). The PSGs, like a number of growth factors, have domain structures very similar to members of the immunoglobulin (Ig) gene superfamily (8–10).

A potential role of the PSGs in hematopoiesis is becoming increasingly apparent. PSG was initially reported to be present in normal polymorphonuclear cells (11, 12). Immunohistochemical studies later showed that PSG is present

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This work was supported in part by NIH grants HD 21793 and HD 31553.

Received April 22, 1997. [P.S.E.B.M. 1998, Vol 217]
Accepted September 28, 1997.

0037-9727/98/2172-0212$10.50/0
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in both granulocytes and monocytes (13). These observations were confirmed by demonstration of the presence of PSG transcripts in both peripheral blood cells and uncultured bone marrow cells using polymerase chain reaction (PCR) (14). The presence of PSGs in first trimester fetal liver but not adult liver (7, 15–18) further suggests the potential role of the PSGs in early hematopoiesis. Preliminary studies demonstrated an enhancing effect of human PSGs on megakaryocyte growth *in vitro*. To corroborate these observations, the present study aims at examining the effect of administration of purified human PSGs on the regeneration of blood cells in γ-irradiated mice given a syngeneic graft.

Materials and Methods

Materials. Crystalline human serum albumin (HSA) was obtained from Calbiochem (La Jolla, CA). Recombinant human IL6 was purchased from R & D Systems, Inc. (Minneapolis, MN). B-D Unopettes were purchased from Fisher Scientific Co. (Columbia, MD). Five to 6-week-old female BalbC/J X A/J F1 (CAF1) mice were purchased from the Jackson Laboratory (Bar Harbor, ME). All mice were kept in sterile cages housed in a laminar flow rack with controlled 12-hr light-dark cycle and fed autoclaved mouse chow and drinking water containing 0.2% oxytetracycline hydrochloride (Sigma, St. Louis, MO) *ad libitum* beginning 1 week prior to irradiation and maintained at this dose throughout the experiment. All protocols involving animals were approved by Georgetown University Animal Care and Use Committee (GUACUC). Pure human pregnancy-specific β1-glycoprotein was obtained from Dr. Hans Bohn at Behringwerke AG, Marburg, FRG, who discovered, purified, and characterized the PSGs. The protein was purified as described previously (19). The same pure protein was used previously to raise polyclonal antibodies that were used to clone the PSG cDNAs from a human cDNA expression library (20).

Bone Marrow Preparation. Bone marrow cells were forced from the tibias and femurs from normal mice with HEM (Hepes-buffered Minimum Essential Medium) containing 5% fetal calf serum. Bone marrow cells were washed three times with HEM by centrifugation at 1000 rpm for 10 min at 4°C and resuspended repeatedly in new media. After the third spin, the cells were resuspended in sterile Dulbecco's phosphate buffered saline (PBS) (GIBCO, Gaithersburg, MD) and kept on ice while cell number was determined with a hemacytometer. Bone marrow samples were diluted 1:1 with toluidine blue in acetic acid for counting white blood cells and diluted 1:1 with trypan blue to determine the percentage of viable cells. An average of 20–30 × 10^6 cells could be obtained from two legs of a mouse.

Sample Preparation. Protein samples were dissolved in sterile Dulbecco's PBS. The solution was filtered using a 0.2 mm Anatop filter (Alltech Assoc., Inc., Deerfield, IL), then aliquoted into appropriate volumes, frozen, lyophilized and stored at −20°C before use. Before injection, the protein aliquots were redissolved with appropriate volumes of filter-sterilized water.

Bone Marrow Transplant and Injection Protocol. The mice were given 1100 cGy from a ^{137}Cs source (Best Industries, Springfield, VA) at Day 1. After 24 hr, each mouse was injected intravenously with 5 × 10^6 viable nucleated bone marrow cells in ≤ 0.5 ml PBS *via* the tail vein (21). This was Day 0. The mice were divided into seven groups. Two mice served as unirradiated controls and were not given bone marrow. Two mice served as irradiation controls and received no bone marrow transplant (BMT). The remaining five groups had five mice in each group. The first group, serving as controls, received 0.1 μg HSA/day; the second group, serving as positive controls, received 0.1 μg IL6/day; the third group received 1 μg PSG/day; the fourth group received 10 μg/day; and the fifth group received 50 μg/day. The body weights of the mice were 18.5 ± 1.2 g on Day 1 of the experiment. Total blood volume of these mice was about 1.2 ml. Therefore, the 1-μg dose would give a blood level of about 1 μg PSG/ml (13.9 n*M*) in the mice. The approximate Molar dose for HSA in the first group was 1.48 m*M*, for IL6 in the second group was 5 n*M*, for 10 μg PSG in the fourth group was 139 n*M*, and for 50 μg PSG in the fifth group was 695 n*M*. The mice were injected with the appropriate volume intraperitoneally (ip). Injections started on Day 1 and were then given daily for 20 days in groups 2 and 5 and for 26 days in the other three groups. Injections were given at about the same time each day and on days when the mice were bled for platelet and WBC counts, injections were done after bleeding. The first bleeding was done on Day 4 and from then on the mice were bled every other day.

Platelet and White Blood Cell Counting. Mice were bled *via* the retro-orbital vein under anesthesia. About 30 μl of blood were drawn into a B-D Unopette coated with sodium EDTA, diluted 1:100 with 1% ammonium oxalate, and mixed thoroughly to prevent clumping of platelets. The white cells and platelets were then counted manually on a Nebauer hemacytometer.

Statistical Analysis. The statistical significance of variance among means was determined using the two-sample student's *t* test assuming equal variances. The two-tailed *P* values are presented and $P < 0.05$ was considered significant unless otherwise stated.

Results

Body Weight and Control Values. Mice were weighed on Day 1 and on Day 14 of the experiment. All mice weighed about the same at the start of the experiment. On Day 14, the weight ranged from 19–21 g with equal distribution of different weights in each group.

The two irradiation control mice died on Day 11 and Day 12 respectively. All other mice survived through the experiment and were apparently healthy. Platelet counts of unirradiated normal control mice ranged from 246,800 to

250,000/μl, and WBC counts ranged from 15,890 to 17,430/μl. These values are comparable to reported values of normal platelet counts of 246–339 × 10^9/l (22) and normal WBC counts of 5–11 × 10^9/l (23).

Platelet Recovery. Results of platelet regeneration are presented in Table I and Figure 1. There was a slow recovery of platelets in all five mice in the HSA group. Starting on Day 8, platelet counts of this group of mice were always significantly ($P < 0.01$) lower than that of the experimental group given 1 μg PSG/day.

The platelet counts of two mice given IL6 also showed faster recovery after BMT. There was overlap between the rest of this group and the animals in Group 1. The mean platelet count of animals of this group on Day 20 (time at which administration of IL6 was terminated) was 101,200 ± 52,505/μl (range 54,400–178,480/μl), which was higher than that of Group 1. This observation was comparable to previous reports that IL6, which has a broad range of effects on hematopoietic stem and progenitor cells and circulating blood cells (24), stimulated platelet production *in vivo* (25).

Platelet count in all animals in the Group 3 mice, which received 1 μg of PSG/day, recovered significantly faster than in animals of any other group. On Day 20, the platelet counts of animals in this group ranged from 162,800–200,200/μl with a mean platelet count of 178,600 ± 15,759/μl. On Day 26, the mean platelet count reached a low normal value of 190,800 ± 6,380/μl with a range of 185,400–200,500/μl. This value was 3-fold higher than that of the control group. On the other hand, both the 10-μg and 50-μg PSG group did not differ from the control group (Table I). The difference in the rate of recovery of platelet counts among the five groups is apparent in Figure 1.

White Blood Cell Recovery. The means and standard deviations of WBC counts of the five treatment groups are presented in Table II. The WBC count of most animals was close to 0 on Day 4 in all groups. The number of WBC of all animals in the control group increased steadily throughout the experimental period and achieved a mean value of 8,750 ± 328/μl (range 8,200–9,000/μl) on Day 26.

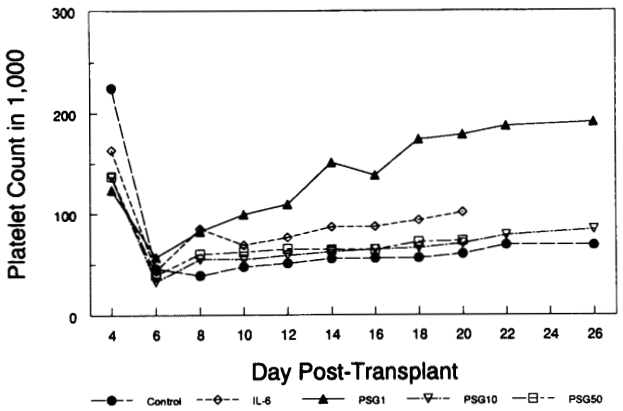

Figure 1. The mean platelet counts of the five groups of mice. The mean platelet counts of each group are plotted. The procedure was the same as described for Table I.

WBC counts of animals in the IL6 treatment group also recovered and achieved similar values to those in the control group. Instead of increasing steadily throughout the treatment period, as observed in the control mice, the WBC counts of this group of mice increased slowly initially. The most significant increment occurred between Day 8 and Day 10.

Mice given either 1 μg PSG/day or 10 μg PSG/day regained normal WBC counts in a significantly shorter period compared to the other groups. Initially, the WBC counts of all groups were comparable. Between Day 4 and

Table I. *In vivo* Effect of PSG on Platelet Regeneration

DAYS (Post-BMT)	4	6	8	10	12	14	16	18	20	22	26
Group 1 (HSA)	225* ± 80	46 ± 15	39* ± 20	48* ± 15	51* ± 16	55* ± 16	56* ± 17	56* ± 16	60* ± 15	69* ± 13	69* ± 15
Group 2 (IL6)	163 ± 18	44 ± 8	84 ± 34	69 ± 30	76 ± 36	8 ± 46	87 ± 46	93 ± 48	101 ± 52	ND	ND
Group 3 (PSG1)	123# ± 80	57 ± 7	82# ± 20	98# ± 21	109# ± 20	151# ± 39	137# ± 58	174# ± 41	179# ± 16	187# ± 24	191# ± 6
Group 4 (PSG10)	137# ± 29	33*# ± 7	55* ± 10	55* ± 10	59* ± 8	62* ± 9	65* ± 9	66* ± 8	70* ± 9	78* ± 9	84* ± 5
Group 5 (PSG50)	137 ± 40	40* ± 6	60 ± 29	62* ± 22	65* ± 19	64* ± 19	64* ± 19	72* ± 23	73* ± 23	ND	ND

Note. Numbers in table are means ± SD × 10^3/μl. There were five mice in each group.
* Indicates significantly different from corresponding values in Group 3 (PSG1) with a two-tailed $P < 0.05$.
# Indicates significantly different from corresponding values in Group 1 (HSA) with a two-tailed $P < 0.05$.
ND Indicates not determined.
Mice were given 1100 cGy whole-body irradiation on Day 1. After 24 hr, each mouse was injected intravenously with 5 × 10^6 viable nucleated bone marrow cells in PBS *via* the tail vein. This was Day 0. The mice were divided into five groups with five mice in each group. The first group received 0.1 μg HSA/day. The second group received 0.1 μg IL6/day. The third group received 1 μg PSG/day. The fourth group received 10 μg PSG/day and the fifth group received 50 μg PSG/day. The injections (ip) started on Day 1, 24 hr after BMT and were then given daily for 20 days for Groups 2 and 5 and for 26 days for the other three groups. The injection was given at about the same time each day and on days when the mice were bled for cell counts, the injection was given after bleeding. The first bleeding was done on Day 4 and from then on, the mice were bled every other day. Mice were bled *via* the retro-orbital vein. About 30 μl of blood were obtained, and platelet counts were determined using a Nebauer hemacytometer.

Table II. *In vivo* Effect of PSG on WBC Count

DAYS (Post-BMT)	4	6	8	10	12	14	16	18	20	22	26
Group 1 (HSA)	2.10 ± 0.06	2.30* ± 0.77	2.32* ± 0.78	4.70* ± 0.99	4.61* ± 1.08	5.86* ± 0.40	6.34* ± 0.89	6.53* ± 1.22	7.28* ± 0.70	7.98* ± 0.91	8.75 ± 0.33
Group 2 (IL6)	1.20 ± 0	1.78 ± 0.27	1.8 ± 0.33	5.02 ± 1.43	5.16 ± 1.46	6.16 ± 1.00	6.07 ± 1.13	6.69 ± 1.00	7.22 ± 0.84	ND	ND
Group 3 (PSG1)	1.68 ± 0.88	9.58# ± 2.79	9.59# ± 2.77	9.88# ± 2.75	10.12# ± 2.86	10.36# ± 2.28	10.73# ± 2.83	11.18# ± 3.34	11.90# ± 3.56	12.46# ± 3.50	12.44# ± 3.68
Group 4 (PSG10)	1.00# ± 0	7.44# ± 2.36	7.40# ± 2.40	8.38# ± 2.67	8.91# ± 2.29	9.77# ± 2.58	10.21# ± 2.43	10.67# ± 2.91	11.27# ± 2.58	11.79# ± 2.52	12.15# ± 3.02
Group 5 (PSG50)	1.15# ± 0.07	1.29*# ± 0.53	1.38* ± 0.53	1.86*# ± 0.46	2.88# ± 0.94	4.54*# ± 0.81	4.67*# ± 0.79	5.30*# ± 0.48	5.64*# ± 0.54	ND	ND

Note. Numbers in table are means ± SD × 10^3/μl. There were five mice in each group.
* Indicates significantly different from corresponding value in Group 3 (PSG1) with a two-tailed $P < 0.05$.
# Indicates significantly different from corresponding value in Group 1 (HSA) with a two-tailed $P < 0.05$.
ND Indicates not determined.
The procedure was the same as described for Table I. WBC counts were determined using a Nebauer hemacytometer under low-power light microscope.

Day 6 post BMT, there was a sharp increase in WBC count in both the 1-μg PSG and the 10-μg PSG group. From then on, WBC count increased steadily until normal values were achieved in these two groups of mice. The WBC counts of mice in these two groups from Day 6 to Day 20 were significantly ($P < 0.05$) higher than those of the other groups. The five mice in the 1-μg PSG group could be divided into two subgroups according to their WBC count recovery profile. Mice 3, 4, and 5 responded faster and achieved a higher value than mice 1 and 2. It was important to note that mice with more rapid recovery of WBC counts did not necessarily have similarly rapid recovery of platelet counts.

In contrast to the mice given 1 μg PSG/day and 10 μg PSG/day, mice given 50 μg PSG/day did poorly, even in comparison with the control group. Similar to that observed for the control mice, there was a steady increase in WBC counts in mice of this group (Table II). However, the increase was very slow and by Day 20, the mean WBC count was significantly ($P < 0.05$) lower than that of the 1-μg PSG and 10-μg PSG groups. The mean WBC counts of the five groups at different times post-BMT are plotted in Figure 2.

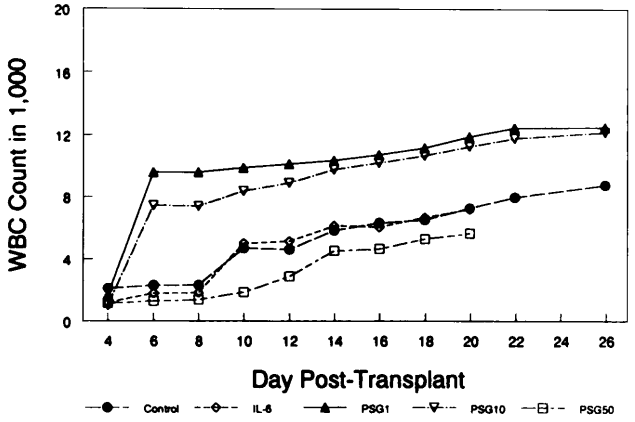

Figure 2. The mean WBC counts of the five groups of mice. The mean WBC counts of each group are plotted. The procedure was the same as described for Table I.

Discussion

The PSG family is composed of a number of highly homologous proteins. Even though the PSG used in the experiments was highly purified, the purification procedure does not allow separation of the different members of the family. Consequently, the results observed were likely to be the combined effect of more than one species of PSG. Further experiments must be performed with homogenous preparations of the different PSG species in order to assign the observed hematopoietic activity to specific PSG(s). Presence of contaminating protein is of a lesser concern since the pure protein was provided by Dr. Hans Bohn (the Bohn protein) and was used to raise polyclonal antibodies that were employed by laboratories worldwide to identify a certain protein to be PSG and from which a number of PSG cDNAs were cloned (20). If a major contaminant was present, the screening procedure should have pulled out cDNAs other than that of the PSGs. All cDNAs cloned by immunological screening of expression libraries using these polyclonal antibodies so far have been shown to be members of the PSG family (5). Neither cytokine nor interleukin cDNA have been cloned using these antibodies indicating that the antigens (i.e., the Bohn PSG preparation) used do not contain a significant amount of contaminating cytokines or interleukins.

What is the biological relevance of studying the action of human PSG on mouse hematopoiesis? Besides being found in humans and subhuman primates, PSGs have also been identified in rodents (reviewed by Refs. 5 and 10). In the mice, pregnancy-associated murine protein-2 (PAMP-2) was found to cross-react with human PSG. The maternal serum levels of PAMP-2 correlate with placental mass and increase with the progression of gestation. PAMP-2 is present in trophoblastic cells in the spongiotrophoblast of pregnant mice. PAMP-2 and human PSG have comparable physiochemical properties (26). Presence of PSGs in the mouse was also confirmed at the transcriptional level (27–29). Expression of a murine PSG mmCGM5 was shown in mouse placenta (28) whereas three murine PSGs were

shown to be present in both spongiotrophoblast of the placenta and the embryo starting at 12.5 days of gestation (29). Similar to that observed with human PSG (30), administration of antibodies against PAMP-2 resulted in prevention of implantation (31). Since murine PSG in many ways is similar to human and subhuman primate PSGs, it is assumed that human PSGs also act on the murine hematopoietic system.

The amounts of PSG used in the experiments reflect the consideration of the presence of multiple species of the protein in the preparation. The molecular mass of HSA is 69 kD (32) whereas that of IL6 is 20–26 kD (33) and that of PSG is 72 kD. Since it is not known which member(s) of the PSG family has the speculated activity and that there are at least 16 different PSGs found in the placenta (5), dosages of 1, 10, or 50 μg of PSG were used in experiments, and 0.1-μg dosages of HSA and IL6 were used as control. The effects of the administration of more protein in the experimental animals given PSG were monitored by comparing the results of the 1-μg PSG group with that of the 10-μg and 50-μg groups. As shown by the results obtained, the increase in platelet regeneration or WBC recovery after the administration of 1 μg of PSG was not due to a dose response since the 50-μg PSG group in most cases behaved similarly to the controls.

It is interesting to note that administration of 50 μg of PSG/day did not enhance platelet and WBC recovery. This might be expected since some members of the PSG family possess immunosuppressive activity. Previous studies showed that PSG suppresses the proliferative activity of phytohemagglutinin-stimulated lymphocytes (34, 35). Thus, depending on the relative amount of the PSGs that possess growth-enhancing activity and those with immunosuppressive activity, the PSG preparation might exhibit opposite effects. It is possible that the immunosuppressive PSGs are present as a minor component in the PSG preparation such that their activity is negligible when only 1 μg of PSG was given. However, at a higher concentration, these PSGs might be toxic or might elicit a feedback mechanism stimulating other cells to produce inhibitory substances resulting in enhancement of neither platelet regeneration nor WBC recovery.

The recovery of WBC counts after BMT was enhanced by the injection of PSG and not with IL6 as previously reported (25). Normal values were achieved in a much shorter time in animals given PSG compared to controls. The response of WBC to injection of PSG could not have been due to endotoxin present in the PSG preparation because mice injected with 50 μg PSG/day did not demonstrate the same response, and WBC counts stayed low throughout the experiment.

The results clearly demonstrate that human PSG hastened the recovery of platelets and WBC after BMT. A survey of the literature reveals an intimate relationship between the PSG family and the hematopoietic system. A number of studies have shown that these proteins are produced by a variety of nonplacental sources including lung, intestine, testis, uterus, fetal liver, submandibular salivary gland, peripheral blood cells, and bone marrow cells (5, 7), all known to be sources of hematopoietic growth factors (1, 36). Studies at both the protein and transcript levels also suggested involvement of the PSGs in blood cell development. PSG was initially reported to be present in normal polymorphonuclear cells (11, 12). Further immunohistochemical studies showed that PSG is present both on the surface and in the cytoplasm of granulocytes whereas lymphocytes do not contain the protein. This presence of PSG in hematopoietic cells was confirmed at the transcript level. Studies with PCR showed that some cells, notably T cells, consistently contained a higher amount of PSG transcript than polymorphonuclear cells, monocytes, and B cells. Besides using uncultured blood cells, a number of studies have also been performed with established blood cell lines. Cultivated blood cells of the myelomonocytic lineage such as KG-1 express PSG, which was detected at both the mRNA (37) and protein (13) levels, with or without stimulation with lipopolysaccharide, phorbol 12-myristate 13-acetate or γ-interferon. This was confirmed by the cloning of PSG cDNAs from HL-60 (38) and KG-1 (39) cell libraries. On the other hand, cells of erythroid lineage, including HEL and K562, and lymphoblastoid cell lines established by Epstein-Barr virus infection of normal B cells do not express PSG before or after induction (13, 37). Recently, a potential receptor for one of the PSG family members, PSG11s, was identified on promonocytic cells (40). The case for a link between the PSG family and the hematopoietic system lies in its presence in the first trimester fetal liver (15–18, 41), the initial anatomic site of hematopoiesis (27). In adults, PSG is present in the bone marrow (14), but not in the liver (5). Furthermore, PSG has also been shown to possess *in vitro* growth-enhancing activities. The observations that PSG is not detectable in tissue slides or uncultured cells but is induced by culturing (32, 42) suggest that it might have an important role in sustaining cell growth. Co-culture studies with transfectants expressing recombinant PSGs further showed that these proteins enhance the growth and maturation of two-cell mouse embryos (43). Altogether, these observations corroborate the results obtained in the present study implicating a role of the PSG in early hematopoiesis in humans.

Although PSGs stimulate thrombopoiesis, they are distinctly different from thrombopoietin (TPO), a well-established natural stimulator of megakaryocytopoiesis (44–46). PSG members vary in size from 58–72 kD (47) whereas recombinant murine TPO purified from serum-free medium has an apparent size of 70 kD, and the processed protein purified from irradiated rat, pig, or canine plasma was reported to be 18–32 kD (48). PSGs are produced by the placenta and a number of nonplacental tissues notably with the exception of liver and kidney (5); TPO is produced by both liver and kidney (48). Lastly, unlike TPO, PSGs have a broad range of effects in *in vitro* systems (34, 35, 38, 49). In this respect, the PSGs might be similar to IL6, which has

also been shown to enhance production of platelets in both mice (25) and cynomolgus monkeys (50). Even though the PSGs, unlike TPO, do not appear to be the major regulator of megakaryocytopoiesis, they might serve to augment the activity of TPO in order to maximize thrombopoiesis and stimulate multiple cell lines. It is known that TPO cooperates with early-acting cytokines, such as interleukin-3 (IL3) and stem cell factor (the c-kit ligand) (SCF), and later-acting cytokines, such as IL6, interleukin-11 (IL11), leukemia inhibitory factor (LIF), and erythropoietin (EPO) (51–54). These hematopoietic growth factors act either synergistically, as in the case of SCF and EPO, or additively as in the case of IL3, with TPO in platelet activation (55). Thus, the PSGs might serve to prime cells or act synergistically with TPO or the other growth factors. They might have a less specific or a wider spectrum of activity during early hematopoiesis compared to TPO.

Even though a heterogeneous mixture of PSGs was used in the present study, demonstration of the hematopoietic growth-enhancing activities of the PSGs has significant implications. Characterization of these activities of the PSG will contribute to the understanding of the development of the hematopoietic system. It will also shed light on the understanding of the change in hematopoiesis during pregnancy. More importantly, defining the biological activities of the PSGs in hematopoiesis might prove these proteins to be useful in *ex vivo* expansion of cord and peripheral blood cells, as an additional tool, or as a component in combination therapy to hasten multilineage hematopoietic recovery after aggressive cytotoxic therapy (56, 57) or in other disorders of bone marrow function.

We would like to thank Dr. Monika Metzger for her help in statistical analysis of the data and Ms. Ann Wright for her help in bleeding mice and counting platelets.

1. Morstyn G, Burgess AW. Hemopoietic growth factors: A review. Cancer Res **48:**5624–5637, 1988.
2. Boehm K, Kelley MF, Ilan J. The interleukin 2 gene is expressed in the syncytiotrophoblast of the human placenta. Proc Natl Acad Sci USA **86:**656–660, 1989.
3. Kameda T, Matsuzaki N, Sawai K. Production of interleukin-6 by normal human trophoblast. Placenta **11:**205–213, 1990.
4. Bohn H. Nachweis und Charakterisierung von Schwangerschaftsproteinen in der menschlichen Plazenta sowie ihre quantitative immunologische Bestimmung im Serum von schwangeren Frauen. Arch Gynaek **210:**440–457, 1971.
5. Chan WY. The pregnancy-specific β1-glycoprotein family. Advances in Contraception Delivery Systems **7:**21–52, 1991.
6. Teglund S, Olsen A, Khan WN, Frangsmyr L, Hammarstrom S. The pregnancy-specific glycoprotein (PSG) gene cluster on human chromosome 19: Fine structure of the 11 PSG genes and identification of 6 new genes forming a third subgroup within the carcinoembryonic antigen (CEA) family. Genomics **23:**669–684, 1994.
7. Teglund S, Zhou G-Q, Hammerstrom S. Characterization of cDNA encoding novel pregnancy-specific glycoprotein variants. Biochem Biophys Res Commun **211:**656–664, 1995.
8. Watanabe S, Chou JY. Isolation and characterization of complementary DNAs encoding human pregnancy-specific β1-glycoprotein. J Biol Chem **263:**2049–2054, 1988.
9. Chou JY, Plouzek CA. Pregnancy-specific β1-glycoprotein. Semin Reprod Endocrinol **10:**116–126, 1992.
10. Thompson JA, Zimmermann W. The carcinoembryonic antigen gene family: Molecular biology and clinical perspectives. J Clin Lab Anal **5:**344–366, 1991.
11. Inaba N, Renk T, Ax W, Schottler S, Weinmann E, Bohn H. Possible synthesis of pregnancy-'specific' beta 1-glycoprotein (SP1) and placenta-'specific' tissue proteins (PP10, PP12) by human and cynomolgus monkey leukocytes. Acta Haematol **66:**35–38, 1981.
12. Inaba N, Renk T, Ax W, Weinmann E, Schottler S, Bohn H. Immunohistochemical detection of pregnancy and placental proteins in human and cynomolgus monkey (*Macaca fascicularis*) peripheral blood cells. Blut **43:**315–325, 1981.
13. Heikinheimo M, Gahmberg CG, Bohn H, Andersson LC. Oncoplacental protein SP1: A constitutive and inducible late differentiation marker of the human myelomonocytic lineage. Blood **70:**1279–1283, 1987.
14. Wu SM, Bazar LS, Cohn ML, Cahill RA, Chan WY. Expression of pregnancy-specific β1-glycoproteins in hematopoietic cells. Mol Cell Biochem **122:**147–158, 1993.
15. Khan WN, Osterman A, Hammarstrom S. Molecular cloning and expression of cDNA for a carcinoembryonic antigen-related fetal liver glycoprotein. Proc Natl Acad Sci USA **86:**3332–3336, 1989.
16. Khan WN, Hammarstrom S. Carcinoembryonic antigen gene family: Molecular cloning of cDNA for a PSG/FL-NCA glycoprotein with a novel domain arrangement. Biochem Biophys Res Commun **161:**525–535, 1989.
17. Zimmermann W, Weiss M, Thompson JA. CDNA cloning demonstrates the expression of pregnancy-specific glycoprotein genes, a subgroup of the carcinoembryonic antigen gene family, in fetal liver. Biochem Biophys Res Commun **163:**1197–1209, 1989.
18. Khan WN, Hammarstrom S. Identification of a new carcinoembryonic antigen (CEA) family member in human fetal liver: Cloning and sequence determination of pregnancy-specific glycoprotein. Biochem Biophys Res Commun **168:**214–225, 1990.
19. Bohn H. Isolation and characterization of placental proteins with special reference to pregnancy-specific β1-glycoprotein and other proteins specific to the placenta. In: Klopper A, Chard T, Eds. Placental Proteins. New York: Springer Verlag, pp71, 1979.
20. Chan WY, Borjigin J, Zheng QX, Shupert WL. Characterization of cDNA encoding human pregnancy-specific β1-glycoprotein from placenta and extraplacental tissues and their comparison with carcinoembryonic antigen. DNA **7:**545–555, 1988.
21. Cohn MJ, Scott DW. Functional differentiation of T-cell precursors. II. Recovery of immunocompetence in lethally irradiated bone marrow reconstituted mice. Thymus **2:**61–74, 1980.
22. Copley AL, Robb TP. Studies on platelets. III. The effect of heparin *in vivo* on the platelet count in mice and dogs. Am J Clin Pathol **12:**563–570, 1942.
23. Bannerman RM. Hematology. In: Foster HL, Small JD, Fox JG, Eds. The Mouse in Biomedical Research. American College of Laboratory Animal Medicine Series. New York: Academic Press, Vol **III:** pp293–312, 1983.
24. Pojda Z, Tsuboi A. *In vivo* effects of human recombinant interleukin 6 on hemopoietic stem and progenitor cell and circulating blood cells in normal mice. Exp Hematol **18:**1034–1037, 1990.
25. Ishibashi T, Kimura H, Shikama Y, Uchida T, Kariyone S, Hirano T, Kishimoto T, Takatsuki F, Akiyama Y. Interleukin-6 is a potent thrombopoietic factor *in vivo* in mice. Blood **74:**1241–1244, 1989.
26. Alper CA. The plasma proteins. In: Williams WJ, Beutler E, Erslev AJ, Lichtman MA, Eds. Hematology, 4th Edition. New York: McGraw-Hill, Inc., pp1616–1627, 1990.
27. Christensen RD. Hematopoiesis in the fetus and neonate. Pediatr Res **26:**531–535, 1989.
28. Hau J. Murine models to human pregnancy-specific β1-glycoprotein

and α-fetoprotein and their application in teratogenic studies. In: Hau J, Ed. Pregnancy Proteins in Animals. New York: Walter de Gruyter, pp465–479, 1986.
29. Rudert F. Isolierung von Mitgliedern der karzinoembryonalen Antigen (CEA)-Genfamilie der Maus und Analyse potentieller Promotor/ Enhancer-Bereiche. Doctoral Dissertation, University of Freiburg, Freiburg, Germany, 1991.
30. Chen DS, Asanaka M, Yokomori K, Wang F-I, Hwang SB, Li H-P, Lai MMC. A pregnancy-specific glycoprotein is expressed in the brain and serves as a receptor for mouse hepatitis virus. Proc Nat Acad Sci USA **92:**12095–12099, 1995.
31. Kromer B, Finkenzeller D, Wessels J, Dveksler G, Thompson J, Zimmermann W. Coordinate expression of splice variants of the murine pregnancy-specific glycoprotein (PSG) gene family during placental development. Eur J Biochem **242:**280–287, 1996.
32. Bischof P. Placental Proteins. In: Keller PJ, Ed. Contributions to Gynecology and Obstetrics. New York: Karger, Vol **12:** pp6–92, 1984.
33. Hau J, Gidley-Baird A, Westergaard JG, Teisner B. The effect on pregnancy of intrauterine administration of antibodies against two pregnancy-associated murine proteins, murine pregnancy-specific beta 1-glycoprotein and murine pregnancy-associated alpha 2-glycoprotein. Biomed Biochem Acta **44:**1255–1259, 1985.
34. Majumdar S, Mapa MK, Gupta AN, Devi PK, Bapna BC, Subrahmanyam D. Inhibitory effect on the blastogenic response to mitogen by placental protein SP-1. Indian J Med Res **73:**751–755, 1981.
35. Harris SJ, Anthony FW, Jones DB, Masson GM. Pregnancy-specific β1-glycoprotein: Effect on lymphocyte proliferation *in vitro.* J Reprod Immunol **6:**267–270, 1984.
36. Heidari Z, Isobe K, Kiuchi K, Goto S, Nakashima I, Tomoda Y. Promotion of proliferation of murine hematopoietic stem cells by growth factors in murine amniotic fluid. J Reprod Immunol **31:**51–64, 1996.
37. Chan WY, Sung SSJ. Differential expression of human pregnancy-specific β1-glycoprotein in erythroid and myeloid cell lines. FASEB J **3:**A323, 1989.
38. Oikawa S, Inuzuka C, Kuroki M, Matsuoka Y, Kosaki G, Nakazato H. A pregnancy-specific β1-glycoprotein, a CEA family member, expressed in a human promyelocytic leukemia cell line, HL-60: Structures of protein, mRNA and gene. Biochem Biophys Res Commun **163:**1021–1031, 1989.
39. Barnett TR, Pickle W II, Elting JJ. Characterization of two new members of the pregnancy-specific β1-glycoprotein family from the myeloid cell line, KG-1, and suggestion of two distinct classes of transcription unit. Biochemistry **29:**10213–10218, 1990.
40. Rutherfurd KJ, Chou JY, Mansfield BC. A motif in PSG11s mediates binding to a receptor on the surface of the promonocyte cell line THP-1. Mol Endocrinol **9:**1297–1305, 1995.
41. Chan WY, Tease LA, Borjigin J, Chan PK, Rennert OM, Srinivasan B, Shupert WL, Cook RG. Pregnancy-specific β1-glycoprotein mRNA is present in placental as well as nonplacental tissues. Hum Reprod **3:**677–685, 1988.
42. Rosen SW. New placental proteins: Chemistry, physiology and clinical use. Placenta **7:**575–594, 1986.
43. Wu SM, Arnold LL, Rone J, Blomberg LA, Trivedi J, Chan WY. The novel effects of recombinant pregnancy-specific β1-glycoprotein on the preimplantation embryo. Mol Biol Cell **5**(Suppl):352A, 1994.
44. Kaushansky K, Broudy VC, Lin N, Jorgensen MJ, McCarty J, Fox N, Zucker-Franklin D, Lofton-Day C. Thrombopoietin, the Mpl ligand, is essential for full megakaryocyte development. Thromb Haemost **92:**3234–3238, 1995.
45. Farese AM, Hunt P, Boone T, MacVittie TJ. Recombinant human megakaryocyte growth and development factor stimulates thrombocytopoiesis in normal nonhuman primates. Blood **86:**54–59, 1995.
46. Levin J. Thrombopoietin: Clinically realized? N Engl J Med **336:**434–436, 1997.
47. Watanabe S, Chou JY. Isolation and characterization of complementary DNAs encoding human pregnancy-specific β1-glycoprotein. J Biol Chem **263:**2049–2054, 1988.
48. Kaushansky K. Thrombopoietin: The primary regulator of platelet production. Blood **86:**419–431, 1995.
49. Repina MA, Blagoslovenskii GS, Gnilevskaia ZU, Ivanova LV. The effect of trophoblast beta-1-glycoprotein on changes in the cellular link of immunity in infected abortion. Akush Ginekol (Mosk) **12:**47–50, 1989.
50. Asano S, Okano A, Ozawa K, Nakahata T, Ishibashi T, Koike K, Kimura H, Tanioka Y, Shibuya A, Hirano T, Kishimoto T, Takaku F, Akiyama Y. *In vivo* effects of recombinant human interleukin-6 in primates: Stimulated production of platelets. Blood **75:**1602–1605, 1990.
51. Papayannopoulou T. Biologic effects of thrombopoietin, the Mpl ligand, and its therapeutic potential. Cancer Chemother Pharmacol **38**(Suppl):S69–S73, 1996.
52. Wun T, Paglieroni T, Hammond WP, Kaushansky K, Foster DC. Thrombopoietin is synergistic with other hematopoietic growth factors and physiologic platelet agonists for platelet activation *in vitro.* Am J Hematol **54:**225–232, 1997.
53. Metcalf D, Hilton D, Nicola NA. Leukemia inhibitory factor can potentiate murine megakaryocytopoiesis *in vitro.* Blood **77:**2150–2153, 1991.
54. Ku H, Yonemura Y, Kaushansky K, Ogawa M. Thrombopoietin, the ligand for the Mpl receptor, synergizes with steel factor and other early acting cytokines in supporting proliferation of primitive hematopoietic progenitors of mice. Blood **87:**4544–4551, 1996.
55. Broudy VC, Lin NL, Kaushansky K. Thrombopoietin (c-Mpl ligand) acts synergistically with erythropoietin, stem cell factor, and interleukin-11 to enhance murine megakaryocyte colony growth and increases megakaryocyte ploidy *in vitro.* Blood **85:**1719–1726, 1995.
56. Metcalf D. Hematopoietic regulators: Redundancy or subtlety? Blood **82:**3515–3523, 1993.
57. Farese AM, Hunt P, Grab LB, MacVittie TJ. Combined administration of recombinant human megakaryocyte growth and development factor and granulocyte colony-stimulating factor enhances multilineage hematopoietic reconstitution in nonhuman primates after radiation-induced marrow aplasia. J Clin Invest **97:**2145–2151, 1996.