Effect of Pregnancy-Specific β1-Glycoprotein on the Development of Preimplantation Embryo (44360)

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Abstract. **The objective of this study is to test the hypothesis that members of the pregnancy-specific β1-glycoprotein (PSG) family enhance the growth and maturation of embryos. cDNA encoding two members of the PSG family, namely PSG1 and PSG3, were expressed in Chinese Hamster Ovary (CHO) cells with the expression vector pHβAPr-1-neo. Two-cell stage mouse embryos were co-cultured in a two-chamber system with CHO cells expressing either recombinant PSG1 (rPSG1) or PSG3 (rPSG3) in the presence and absence of neutralizing PSG antibodies. The cleavage and maturation stage of the embryos was assessed at 12-hr intervals. Mouse embryos co-cultured with transfectants expressing rPSG1 showed a significant enhancement of cleavage and maturation rate compared to controls with $P < 0.005$–0.004. In co-cultures with CHO cells expressing rPSG3, no significant difference from the controls was observed in the early stage of development until late blastocyst formation. At that stage, there was a statistically significant enhancement of development by rPSG3 when compared to controls with $P < 0.001$. These results suggest that PSG1 and PSG3 exhibit embryotropic activity at different stages of development in the mouse model.**

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Pregnancy-specific β1-glycoproteins (PSGs) were isolated from human pregnancy sera and placental extracts in the early 1970s (1, 2). Initially, PSGs were believed to be synthesized only by the placental syncytiotrophoblast. However, subsequent studies have shown that PSGs were also synthesized by a number of nonplacental tissues (reviewed in Ref. 3) including the testis (4), and the intestine (5) as well as cultured cells including fibroblasts (6) and the hematopoietic cells (7, 8). Molecular genetic studies revealed that the PSG family is composed of 11 highly homologous genes clustered on the long arm of chromosome 19 (9). These genes give rise to a number of differentially spliced products. So far, 16 different PSG cDNAs have been isolated from human placenta (3). The major species of PSG in the human placenta include glycoproteins of 72, 64, and 58 kDa respectively (10). These proteins have domain structures very similar to the carcinoembryonic antigens (CEA) and to other members of the immunoglobulin (Ig) gene superfamily (10–12). The deduced amino acid structures of the PSGs indicate that the majority of them are secreted glycoproteins typically consisting of a 34-amino acid leader sequence, an IgV-like N-domain, one or two IgC-like A-domain, an IgC-like B-domain, and a variable carboxyl terminal C-domain.

A role of PSG in preimplantation embryo development appears apparent. PSG has been detected in the culture media of human preimplantation embryos (13). The protein was secreted at all stages of embryonic development, the majority being found at the 2- to 8-cell stage. We have also detected transcripts encoding PSGs in human endometrial tissue (14). A high level of expression of the PSGs in early pregnancy (6) further suggests potential activity during early embryonic development. We hypothesize that some members of the PSG family also play the critical role of

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messengers between the early embryo and the endometrial tissue. The synthesis of PSGs by the endometrial tissue and the potential growth-enhancing activity support an embryotrophic role of PSGs in early stages of preimplantation development, whereas the very early release of PSG from embryonic cells implicates an autocrine role of the proteins. The proteins may also serve a paracrine role in synchronizing the development of the endometrium and the embryo. Utilizing transfectants expressing recombinant human PSG, studies were designed to test these potential effects of the PSGs in co-culture of mouse embryos.

Materials and Methods

Construction of Expression Plasmid. The expression vector was a generous gift from Dr. Laura J. F. Hefta of the Beckman Research Institute of the City of Hope (Duarte, CA). This vector has been used successfully to express CEA and NCA (nonspecific cross-reacting antigen), two proteins highly homologous to the PSGs (12), in Chinese Hamster Ovary-K1 (CHO) cells (15). The expression plasmid for PSG1 (PSG1/pHβ) was constructed by inserting the 1.3-kb Eco R1 fragment containing the entire coding sequence of the cDNA insert of clone hPS11 (16) into pHβAPr-1-neo. The expression plasmid for PSG3 (PSG3/pHβ) was constructed by inserting the 2.0-kb cDNA insert of clone hPS173 (5) into the expression vector. The expression vector gave an additional 500 bp to the 3′ untranslated region of the transcript of the inserted DNA.

Generation of Permanent Transfectants. Chinese Hamster Ovary-K1 cells (ATCC CCL 61) were grown in Ham's F12 medium supplemented with 10% FBS (fetal bovine serum) and 1% antibiotic/antimycotic solution (all from GIBCO/BRL, Gaithersburg, MD). The cells were cultured at 37°C in 5% CO_2/95% air. Culture medium was changed twice weekly.

CHO cells were transfected with either PSG1/pHβ, PSG3/pHβ or pHβAPr-1-neo by lipofection as described previously (15). 5×10^5 cells were transfected with 20 μg of linearized PSG construct or vector DNA in a T-75 flask. Forty-eight hours after transfection, the cells were selected with 10 ml of Ham's F12 medium supplemented with 10% FBS, 1% antibiotic/antimycotic, 2 m*M* glutamine and 1 mg/ml G418 (Geneticin) (GIBCO/BRL). The cells were grown in this selection medium for 10 days. G-418 resistant CHO transfectants were tested for expression of PSG by nested polymerase chain reaction. Northern blot, and Western blot analyses. Permanent transfectants expressing recombinant PSGs (rPSG1 and rPSG3) were cloned by limiting dilution, expanded, and stocked. The same transfectant was used in all experiments.

Reverse Transcription-Polymerase Chain Reaction (RT-PCR), Nested PCR, Northern Blot, and Western Blot Analyses. Expression of rPSG by the transfectants was first confirmed by RT-PCR using PSG universal primers and PSG gene-specific primers as described previously (8, 14). Controls for amplification efficiency of the RT-PCR and functionality of the RNA preparations were provided by simultaneous addition to the reaction mixture of the primers for PSG and those for glyceraldehyde-3-phosphate dehydrogenase (G3PDH) (17). The G3PDH primers amplified a 161-bp cDNA fragment.

Expression of rPSG1 and rPSG3 was also confirmed by Northern blot and Western blot analyses. Transfectants were cultured to confluence in F12 medium. The confluent monolayer of cells was rinsed twice with Hanks' balance salt solution (HBSS) to remove culture medium. The culture medium was changed to serum-free MCDB media (Sigma Chemical Company, St. Louis, MO) for 24 hr. Cells were harvested for Northern blot analysis. Spent medium was collected every 2–3 days. After removal of dead cells and debris by brief centrifugation at 1,500 rpm for 10 min at 4°C, spent medium was concentrated by lyophilization and used for Western blot analysis. For Northern blot analysis, 100 μg of total cellular RNA extracted with 4 *M* guanidine monothiocyanate-phenol-chloroform (18) were used, and the blot was hybridized with labeled PSG1 or PSG3 cDNA as described previously (19). Control was provided by total cellular RNA extracted from CHO cells transfected with the vector alone. Twenty μg of spent medium protein were used in Western blot analysis. Spent medium of cultures of untransfected CHO cells and CHO cells transfected with unmodified vector were used as controls. Western blots were developed with polyclonal PSG antibodies and revealed by a horseradish-peroxidase-linked second antibodies (Enhanced Chemiluminescence [ECL] detection system, Amersham, Arlington Heights, IL) (20). Exposure time for the blots was 1 min. Positive control was provided by 5 μg of placental protein. Levels of rPSG produced by the cloned permanent transfectants were measured by ELISA as described previously (21).

Embryo Recruitment. The use of animals in this study was approved by the Georgetown University Animal Care and Use Committee. Female CB6F1 mice 6–8 weeks of age (Harlan Sprague-Dawley, Indianapolis, IN) were superovulated by intraperitoneal injection (ip) of 5 IU pregnant mares' serum gonadotropin (PMSG) (Sigma Chemical Company) followed by an ip injection of human chorionic gonadotropin (hCG) (Sigma Chemical Company) 48 hr later. Following hCG injection, females were placed in cages with male studs and allowed to mate overnight. About 40 hr post-hCG injection, the female mice were sacrificed by carbon dioxide asphyxiation, oviducts were excised, and embryos were flushed from the oviducts as described previously (22). Two-cell embryos identified under a dissecting microscope and selected from several mice were randomly divided among different treatment groups.

Co-Culture Experiment. Polyclonal anti-human PSG antibodies were purchased from Boehringer Mannheim Biochemicals (Indianapolis, IN). These antibodies recognize all major species of human PSG as well as some nonhuman PSGs as shown by Western blot analyses (23).

The titer measured approximately 0.03 mg human PSG/ml as given by the manufacturer. Sodium azide in the antibody solution was removed by ultrafiltration and rinsing with phosphate buffered saline (PBS) with a Centricon-100 (Amicon, Beverely, MA). After washing 3 times with 3-fold its original volume of PBS, the antibody solution was reconstituted to its original volume with PBS, and the resulting solution was sterilized by filtration through a 0.5 μm Acrodisc (Gelman Sciences, Ann Arbor, MI). The cytotoxicity of the antibody solution was removed whereas the activity of the antibodies was not affected by this manipulation.

Approximately 2×10^4 CHO cells transfected with the unmodified vector, expressing rPSG1 or expressing rPSG3, were added to each well in a 24-well plate (Costar, Cambridge, MA). The cells were cultured at 37°C for 4 days in Ham's F12 medium supplemented with 10% FBS (0.8 ml medium/well). The cells were then washed once and cultured in serum-free MCDB medium for 24 hr. The medium was removed, and new MCDB medium with or without PSG antibodies (8 μl) was added. After 24 hr, clear transwell inserts of 8.5 mm diameter with 3.0 μm pores (Costar, Cambridge, MA) were placed in the center of the wells and 8–10 two-cell mouse embryos were introduced to each insert. Co-cultures were incubated at 37°C in an atmosphere with 5% CO_2. The developmental stage of the embryos was assessed every 12 hr under light microscopy. Each plate was scored blindly and independently by three of the investigators, and the mean number of embryos in each developmental stage was calculated.

All treatment groups were done in triplicate in an experiment. The experimental treatment groups consisted of co-culturing of embryos with transfectants expressing rPSG1 [rPSG1] or rPSG3 [rPSG3]. Three control treatment groups were included in all experiments. Co-culture with CHO cells transfected with unmodified vector [Vect Cont] provided the control for the effects contributed by CHO cell proteins. Addition of PSG antibodies to this culture [Cont + Ab] provided the control for the effects of the antibodies on the embryos. Antibodies with 1:100 dilution were used. Addition of PSG antibodies to co-cultures with permanent transfectants expressing rPSG1 [rPSG1 + Ab] or rPSG3 [rPSG3 + Ab] would neutralize the effect of the PSG on the embryos and confirm that the effect observed in the co-cultures with PSG-expressing permanent transfectants was specifically due to the effect of rPSG1 or rPSG3. Two dilutions of PSG antibodies, namely 1:100 and 1:200, were tried. Similar results were obtained with antibodies at either 1:100 or 1:200 dilution indicating that the amount of anti-PSG IgG in 8 μl solution at 1:200 dilution was sufficient to cause complete immunoneutralization of the activities of the rPSG.

Statistical Analysis. Statistical analyses were performed using z test for comparison of proportions and by chi-square contingency table analysis. The percentage of embryos at a specific developmental stage was compared among the different treatment groups at the same single time period. Bonferroni-type correction was applied for multiple comparison. The most conservative result was reported.

Results

Expression of rPSG1 and rPSG3. CHO cells transfected with PSG1/pHβ or PSG3/pHβ produced transcripts, which upon RT-PCR using univeral primers gave an amplified fragment of 854 bp (results not shown). When PSG gene-specific primers were used in nested PCR, both PSG1-specific primers and PSG3-specific primers primed the amplification of a 768-bp fragment (Fig. 1A, Lanes P1 and P2, respectively). These amplified fragments were of the size predicted to be produced using transcripts encoded by PSG1 or PSG3 as template (8), suggesting that the transfected hPS11 cDNA and hPS173 cDNA were expressed. No amplified DNA was observed in controls using untransfected CHO cells' total cellular RNA as templates (Figure 1A, Lanes C1 and C2, respectively). The presence of PSG1 and PSG3 transcripts in the transfectants was also confirmed by Northern blot analysis. Hybridizing bands of appropriate size for PSG1 and PSG3 transcripts were demonstrated in transfectants (Figure 1B, Lanes P1 and P2). The control showed no hybridizing transcript (Figure 1B, Lane C).

Expression of rPSGs was also confirmed by Western blot analysis. Positive control was provided by 5 μg of placental protein (Figure 1C, Lane hP). No cross-reacting material was detected in the media of CHO cells transfected with unmodified vector (negative control) (Figure 1C, Lane C) indicating that CHO cells did not synthesize or secrete any protein that cross-reacted with the PSG antibodies. Spent medium of the PSG1 transfectant contained a 72-kDa protein whereas the PSG3 transfectant produced a major protein of 64 kDa cross-reacting with the PSG antibodies (Figure 1C, Lanes P1 and P2, respectively). The amounts of recombinant protein secreted into the medium by the permanent transfectants were determined to be between 5.9–9.6 ng/ml MCDB medium/24 hr with an average of 7.3 ng/ml by radioimmunoassay for both rPSG1 and rPSG3.

Embryotrophic Effect of rPSG1. Two-cell mouse embryos co-cultured with transfectants expressing rPSG1 consistently showed rapid development into morulae and blastocysts. On the contrary, embryos in control co-cultures demonstrated slower development. The results are presented in Table I. The means and standard deviations of triplicate determinations in three independent experiments were presented. Embryos that degenerated were also represented (Degen). At 12 hr (Table IA), 82 ± 4% of the embryos in the [rPSG1] treatment group reached the 4–6-cell stage whereas at most 54% of the embryos in the control groups ([Vect Cont], [Cont + Ab], and [rPSG1 + Ab]) reached a similar stage of development. The difference in the percentage of embryos at the 4–6-cell stage between the [rPSG1] group and each one of the three control treatment

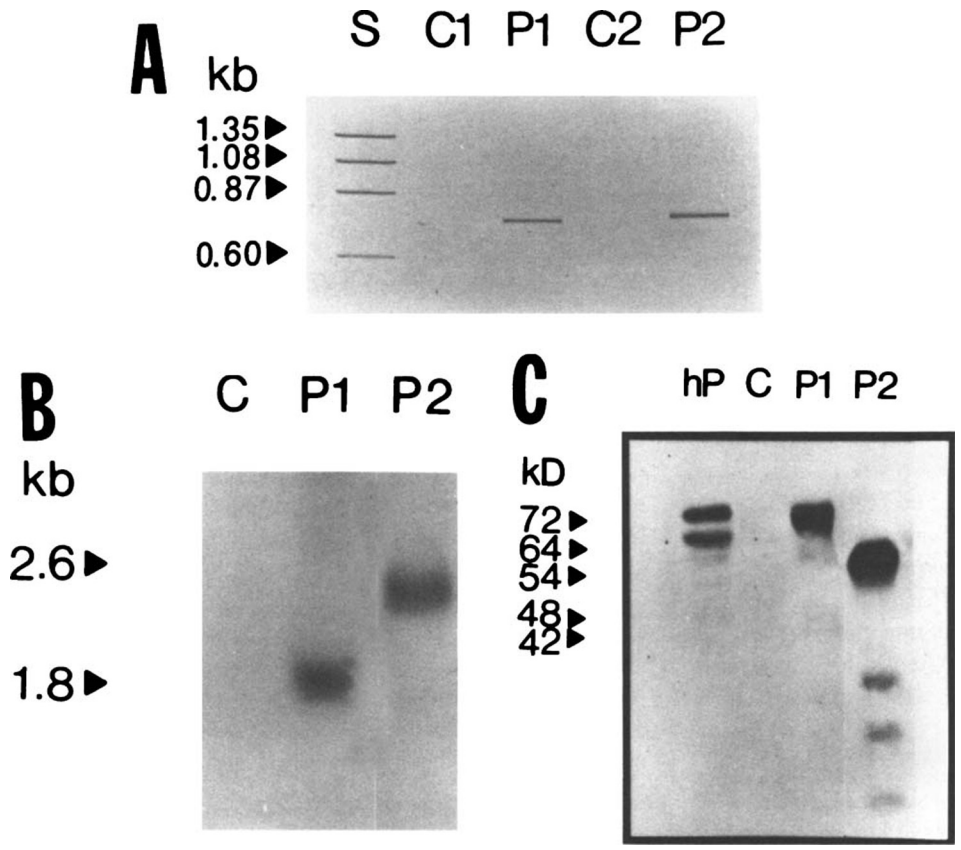

Figure 1. *In vitro* expression of PSG1 and PSG3 in CHO Cells. C, C1, and C2: CHO cells transfected with unmodified vector, negative control; P1: CHO cells transfected with PSG1/pHβ construct; P2: CHO cells transfected with PSG3/pHβ construct. (A) RT-PCR products generated with gene-specific primers. In C1 and P1, the PCR was primed with PSG1-specific primers whereas in C2 and P2 the PCR was primed with PSG3-specific primers. Size of the DNA markers are indicated on the left. (B) Northern blot analysis of total cellular RNA. Size of the RNA hybridizing bands in kb are indicated on the left. (C) Western blot analysis of proteins secreted into the culture medium. Twenty μg of spent medium protein of the different cultures were analyzed. hP represents 5 μg of human placental extract. The molecular mass of the protein standards in kDa are indicated on the left.

groups was significant at $P < 0.0004$. At 24 hr (Table IA), 6% ± 4% of the embryos in the [rPSG1] treatment group reached the morula stage whereas none of the embryos in the control groups reached this stage of development. Furthermore, only 16%–24% of the embryos in the control groups were at the 8–16-cell stage whereas 37% ± 7% of the embryos in the [rPSG1] group were at that stage of development. There was a significant difference ($P < 0.005$) in the percentage of embryos at the early blastocyst stage at 36 hr (Table IA) in the [rPSG1] gruop (26% ± 1%), compared to all the control treatment groups, namely [rPSG + Ab] (3% ± 0%), [Vect Cont] (8% ± 7%), and [Cont + Ab] (5% ± 1%). At 60 hr (Table IB), 94% ± 4% of the embryos co-cultured with PSG-secreting transfectants developed to the late blastocyst stage, whereas presence of PSG antibodies in the media reduced the percentage to only 59% ± 8% ($P < 0.0001$). In the vector control co-cultures, only 55% ± 25% and 68% ± 9% in the absence and presence of PSG antibodies, respectively, developed to the same stage. A number of embryos in the control groups also degenerated during the course of the experiment. On the other hand, degeneration of embryos in the experimental group did not occur until the 72nd hr (Table 1B). The results from this investigation indicated that rPSG1 stimulated early embryonic development *in vitro*.

Embryotrophic Effect of rPSG3. In co-cultures with CHO cells expressing rPSG3, no significant difference from all three control groups was observed in the early stage of development until late blastocyst formation (Table IIA). At 60 hr (Table IIB), 23% ± 5% of the embryos cultured in the presence of rPSG3 ([rPSG3] group) reached the late blastocyst stage compared to only 8% ± 4% in the presence of neutralizing antibodies ([rPSG3 + Ab] group) ($P < 0.005$) and 2% ± 0% in the [Vect Cont] group ($P < 0.005$). At 72 hr (Table IIB), 63% ± 3% of the embryos in the [rPSG3] group were at the late blastocyst stage whereas only 33% ± 5% ($P < 0.005$) were at that stage of development in the [rPSG3 + Ab] group. This was comparable to that observed in the other control groups ([Vect Cont] 27% ± 0%, $P < 0.005$, and [Cont + Ab] 23% ± 12%, $P < 0.005$). After 84 hr (Table IIC), 87% ± 2% of embryos in the [rPSG3] group reached late blastocyst stage whereas less than 50% of the embryos in all control groups were at that stage of development ($P < 0.001$).

Discussion

This is the first report of the embryotrophic activity of human PSGs. Results obtained suggest a stimulatory effect of two members of the PSG family, namely PSG1 and PSG3, on embryogenesis in preimplantation embryos *in vitro*. Maturation was enhanced whereas degeneration was decreased when 2-cell mouse embryos were co-cultured with transfectants producing a single species of recombinant human PSG. The fact that PSG antibodies could block the effect of either of the rPSGs suggests that the activity of the PSG is specific, and the enhancement of embryo growth is not simply due to an increase in protein content of the culture media.

Table I. Effect of rPSG1 on the Development of Mouse Embryos

Stage of development	12th hr				24th hr			
	[Vect Cont][a]	[Cont + Ab]	[rPSG1 + Ab]	[rPSG1]	[Vect Cont]	[Cont + Ab]	[rPSG1 + Ab]	[rPSG1]
2 Cells	20 ± 4[b]	28 ± 1	27 ± 4	5 ± 1				
3 Cells	26 ± 3	24 ± 4	32 ± 10	13 ± 4	10 ± 4	6 ± 7	1 ± 1	1 ± 1
4/6 Cells	54 ± 6[c]	48 ± 3[c]	41 ± 6[c]	82 ± 4[d]	67 ± 12	69 ± 17	71 ± 23	56 ± 0
8/16 Cells					16 ± 14	18 ± 6	24 ± 16	37 ± 7
Morula								6 ± 4
Early blastocyst								
Late blastocyst								
Hatching								
Degeneration					6 ± 4	7 ± 5	4 ± 6	
	36th hr				48th hr			
3 Cells	2 ± 2	2 ± 2		1 ± 1		3 ± 4	2 ± 0	1 ± 1
4/6 Cells	14 ± 6	7 ± 5	17 ± 13		1 ± 2[b]		9 ± 2	2 ± 1
8/16 Cells	12 ± 10	6 ± 6	9 ± 1		4 ± 1	5 ± 7	3 ± 4	1 ± 1
Morula	58 ± 1	78 ± 10	71 ± 16	73 ± 1	56 ± 9	51 ± 4	56 ± 11	50 ± 9
Early blastocyst	8 ± 7[e]	5 ± 1[e]	3 ± 0[e]	26 ± 1[f]	33 ± 9	39 ± 11	26 ± 13	46 ± 9
Late blastocyst								
Hatching								
Degeneration	6 ± 4	2 ± 3			6 ± 1	2 ± 3	4 ± 5	
	60th hr				72nd hr			
3 Cells		1 ± 2	1 ± 1			2 ± 4		
4/6 Cells			3 ± 5			1 ± 2	2 ± 2	1 ± 1
8/16 cells			11 ± 16				3 ± 4	
Morula	5 ± 7	3 ± 1	4 ± 5	1 ± 1				
Early blastocyst	37 ± 20	25 ± 8	18 ± 15	5 ± 2	12 ± 17	5 ± 6	14 ± 20	4 ± 2
Late blastocyst	55 ± 25[g]	68 ± 9[g]	59 ± 8[g]	94 ± 4[h]	84 ± 11	87 ± 3	80 ± 14	93 ± 4
Hatching					1 ± 1	3 ± 1		1 ± 1
Degeneration	3 ± 4	3 ± 4	4 ± 5		3 ± 5	2 ± 3	3 ± 4	1 ± 1

[a] Approximately 2×10^4 CHO cells, transfected with the unmodified vector or rPSG1 expression plasmid, were cultured in each well in a 24-well plate for 4 days. The cells were then washed once and cultured in serum-free MCDB medium for 24 hr. The medium was removed, and new MCDB medium with or without PSG antibodies (8 µl) was added. After 24 hr, clear transwell inserts of 8.5 mm diameter with 3.0 µm pores were placed in the center of the wells, and 8–10 two-cell mouse embryos were introduced to each insert. Co-cultures were incubated at 37°C in an atmosphere with 5% CO_2. The developmental stage of the embryos was assessed every 12 hr under a light microscope. Each plate was scored blindly and independently by three of the investigators, and the mean number of embryos in each developmental stage was calculated. The four treatment groups of embryo co-culture were: (1) [Vect Cont]: CHO cells transfected with unmodified vector ($n = 73$); (2) [Cont + Ab]: Vect Cont CHO cells in the presence of PSG antibodies (1:100 dilution) ($n = 83$); (3) [rPSG1 + Ab]: CHO cells expressing rPSG1 in the presence of PSG antibodies (1:100 dilution) ($n = 82$); (4) [rPSG1]: CHO cells expressing rPSG1 ($n = 153$). n is the total number of embryos in each treatment group. All treatment groups were done in triplicate in an experiment.

[b] The percentage of embryos at each stage of development within each treatment group. The means and standard deviations of triplicate determinations in three independent experiments were presented.

[c] Significantly different from [d] in the same period, $P < 0.0004$

[e] Significantly different from [f] in the same period, $P < 0.005$

[g] Significantly different from [h] in the same period, $P < 0.0001$

Similar to other members of the PSG family, PSG1 and PSG3 are highly homologous. With the exception of the C-domain, the protein encoded by the two genes share >92% of their amino acids (3, 11). In spite of this high homology, there are some subtle structural differences between the two PSGs. PSG1 has a short C-domain of only 5 amino acids, whereas PSG3 has a 14-amino acid C-domain (3). PSG1 has seven potential N-glycosylation sites, whereas PSG3 has only four (11). Consequently, PSG1 is more heavily glycosylated. This presumably gave rise to the difference in size between rPSG1 and rPSG3 demonstrated in Western blot analysis (Figure 1C). The significance of the difference in the number of potential protein kinase C sites between the two PSGs is not clear (11). However, of particular interest is the presence of the Arg-Gly-Asp (RGD) tripeptide in PSG3 but not in PSG1. The RGD sequence has been shown to serve as a cell attachment site, recognized by cell surface receptors, in a number of proteins (24). Such an RGD recognition system is suggested to give positional cues, as well as growth and locomotive signals, to particular cell types in cellular differentiation and migration during development (24). It is tempting to speculate that PSG1 serves in promoting maturation of the embryo in the early phase of development whereas PSG3, with the presence of the RGD sequence, serves in preparing the embryo for attachment to the uterine wall and implantation.

Table II. Effect of rPSG1 on the Development of Mouse Embryos

Stage of development	12th hr				24th hr			
	[Vect Cont][a]	[Cont + Ab]	[rPSG3 + Ab]	[rPSG3]	[Vect Cont]	[Cont + Ab]	[rPSG3 + Ab]	[rPSG3]
2 Cells	3 ± 1[b]	3 ± 1	2 ± 1	4 ± 1				
3 Cells	17 ± 2	10 ± 1	13 ± 4	17 ± 7	4 ± 1			1 ± 0
4/6 Cells	80 ± 5	87 ± 3	85 ± 2	79 ± 3	21 ± 2	11 ± 0	2 ± 1	4 ± 1
8/16 Cells					50 ± 3	59 ± 9	58 ± 2	54 ± 3
Morula					25 ± 4	30 ± 9	40 ± 0	41 ± 5
Early blastocyst								
Late blastocyst								
Hatching								
Degeneration								

	36th hr				48th hr			
3 Cells								
4/6 Cells	3 ± 1							
8/16 Cells	13 ± 0	23 ± 12	8 ± 1	14 ± 12	1 ± 0[b]			
Morula	65 ± 1	63 ± 10	50 ± 0	41 ± 1	55 ± 3	53 ± 23	38 ± 12	28 ± 23
Early blastocyst	19 ± 3	14 ± 2	42 ± 1	45 ± 14	44 ± 2	47 ± 23	62 ± 12	72 ± 23
Late blastocyst								
Hatching								
Degeneration								

	60th hr				72nd hr			
3 Cells								
4/6 Cells								
8/16 cells								
Morula	27 ± 8	19 ± 1	18 ± 6	3 ± 0	7 ± 4	5 ± 2	8 ± 4	
Early blastocyst	70 ± 5	74 ± 9	74 ± 2	74 ± 2	65 ± 8	72 ± 7	59 ± 17	35 ± 2
Late blastocyst	2 ± 0[c]	7 ± 3[c]	8 ± 4[c]	23 ± 5[d]	27 ± 0[c]	23 ± 12[c]	33 ± 5[c]	63 ± 3[d]
Hatching								
Degeneration	1 ± 0				1 ± 0			2 ± 0

	84th hr			
3 Cells				
4/6 Cells				
8/16 Cells				
Morula	1 ± 1[b]		2 ± 0	
Early blastocyst	50 ± 21	40 ± 7	48 ± 2	11 ± 3
Late blastocyst	46 ± 28[e]	48 ± 7[e]	47 ± 3[e]	87 ± 2[f]
Hatching				
Degeneration	3 ± 3	12 ± 0	3 ± 1	2 ± 0

[a] The experiment was the same as that described in Table I except that permanent transfectants expressing rPSG3 were used instead of those expressing rPSG1. The four groups of embryo co-culture were: (1) [Vect Cont]: CHO cells transfected with unmodified vector ($n = 77$); (2) [Cont + Ab]: Vect Cont CHO cells in the presence of PSG antibodies ($n = 36$); (3) [rPSG3 + Ab]: CHO cells expressing rPSG3 in the presence of PSG antibodies ($n = 41$); (4) [rPSG3]: CHO cells expressing rPSG3 ($n = 81$). n is the total number of embryos in each treatment group.
[b] The percentage of the embryos at each stage of development within each treatment group. The means and standard deviations of triplicate determinations in two independent experiments were presented.
[c] Significantly different from [d] in the same period, $P < 0.005$
[e] Significantly different from [f] in the same period, $P < 0.001$

Besides being found in humans and subhuman primates, PSGs have also been identified in rodents (3, 12). In the mice, pregnancy-associated murine protein-2 (PAMP-2) was found to cross-react with human PSG and have comparable physiochemical properties (25). PAMP-2 is present in trophoblastic cells in the spongiotrophoblast of pregnant mice. The maternal serum levels of PAMP-2 correlate with placental mass and increase with the progression of gestation. Intrauterine administration of antibodies against PAMP-2 was found to result in prevention of implantation (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 the spongiotrophoblast of the placenta and the embryo starting at Day 12.5 of gestation (29). Thus, similar to PSG in human and subhuman primates, murine PSG might play an important role in embryonic development.

The physiological activity of the PSGs during pregnancy still remains unknown. Structural homology of the PSGs to the CEA family members led investigators to suggest a cell adhesion role for the PSGs. So far there is no experimental evidence supporting this speculation. A number of earlier studies suggest potential interaction between PSGs and the maternal immune system (6, 30–32). The identification of specific binding of purified placental PSG by phorbol ester–activated human peripheral blood mononuclear cells (33) lends further support to this immunomodulatory activity of the PSGs. More recently, examination of PSG gene expression pattern in endometrial tissue of recurrent aborters suggests the potential contribution of one of the PSGs to the local modulation of the inflammatory T_H1 response in the endometrium of these women (14). Another potential activity of the PSGs is growth promotion. PSG appears to be associated with growth and maintenance of nontransformed cells *in vitro* (34). Human placental PSG preparations were also found to enhance blood cell regeneration after bone marrow transplantation in mice (35). Thus, different PSG family members could be involved in the growth and developmental process.

A number of studies have shown that mammalian embryonic development in culture is associated with retardation of cleavage, species-specific cleavage arrest, and reduction of viability (36). Length of culture time is associated with a developmental block or loss of viability (37, 38). Co-culture of preimplantation embryos with uterine and oviductal epithelial cells abolishes the *in vitro* developmental block (39). Such beneficial effect of co-culture on embryonic development has been observed in various mammalian species such as human, pig, goat, cow, and mouse (40). However, the actual role of the co-culture system is not clear. It might involve embryotrophic factors released by cells or metabolizing embryotoxic substances from the culture media. Also the production of proteins by feeder cell monolayers and cell-to-embryo contact have been cited as potential factors in co-culture support of embryonic development (38). The controls incorporated in the experiments described in the present report precluded CHO cell proteins as factors enhancing embryonic maturation. Results obtained with the [rPSG + Ab] group also precluded increased protein concentration in the culture media as a factor. Cell-to-embryo contact was also not a factor since co-culture was done in a two-chamber system. Thus the embryotrophic effect observed in the experiments was due to the rPSGs secreted by the transfectants.

A number of polypeptide growth factors and cytokines have been identified in the human reproductive tract tissues (41–47). The early embryo also expresses these growth factors and growth factor receptors (48–50). The autocrine, juxtacrine, and paracrine actions of these growth factors, as well as other proteins (51), might be potentially important in cellular proliferation and cell-to-cell communication within the developing embryo, in the reproductive tract, and at the maternal-trophoblast interface. Studies have shown that adequate embryo-endometrial interaction depends on synchronization of the embryo's developmental stage with endometrial maturation (52, 53). Compared to the growth factors, the evidence for a role of the PSGs in embryo-maternal communication is equally or even more compelling. PSG production by the reproductive tract tissues and the developing embryos are well established. Expression of different members of the PSG family was demonstrated in endometrial tissues of fertile reproductive women during the peri-implantation period (14). PSG expression in the peri-implantation endometrium may interact with the embryo from the earliest stages of development. This is quite likely since human embryos had been shown to secrete PSG into the culture medium *in vitro* from Days 3 and 4 after fertilization. Cumulative PSG concentrations secreted by human blastocysts in culture for 14 days were found to be significantly higher than those secreted by vacuolated morulae (54). Similarly, culture baboon embryos were also found to express PSGs starting 4 to 7 days post-attachment to the culture dish (55). This very early secretion of PSG implies juxtacrine, paracrine, or autocrine activities of these proteins in the development of the embryo. Furthermore, it had been shown that those embryos that give rise to successful pregnancies *in vitro* are associated with production of PSG in the culture medium (56). The blockage of PSG embryotrophic activity by antibodies, as shown in the present report, provides the evidence that PSGs act on and enhance the development of the preimplantation embryo. Aberrant expression of the PSGs may lead to breakdown of communication between the embryo and the endometrial tissues and underdevelopment of the embryo. This may be another mechanism by which aberrant expression of some members of the PSG family leads to pregnancy failure as observed in women with recurrent spontaneous abortion (14).

Establishing a primary role for the individual PSGs during early pregnancy requires further experiments documenting the presence of specific PSG receptors on the embryo and/or decidual cell surface. Further elucidation of the molecular control mechanisms for PSG gene expression, as well as the role of each member of the family plays in early embryonic development, could help construct a more complete picture of this process as well as better understanding of possible causes of early pregnancy failure. Furthermore, the development of *in vitro*-fertilized human embryos in the presence of PSG will be an attractive approach to improving embryo viability and pregnancy rates.

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